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Ethanol Preference in Peromyscus: An Association with Compromised Ethanol Metabolism

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ETHANOL PREFERENCE IN PEROMYSCUS; AN ASSOCIATION WITH COMPROMISED ETHANOL METABOLISM

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This thesis is submitted in partial fulfillment of

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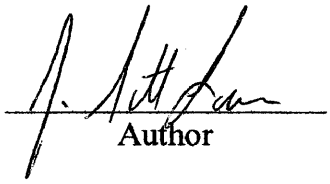

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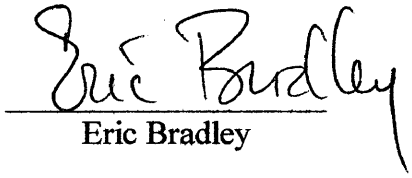
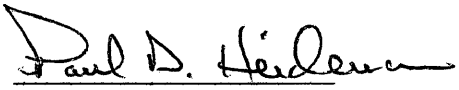
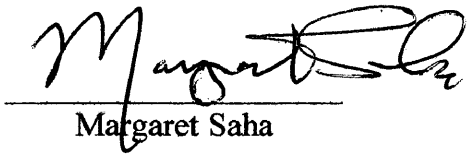


Eric Bradley



Paul Heideman



Margaret Saha

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ABSTRACT

In this study, outbred *P. leucopus* were initially subjected to an ethanol preference test to determine if differences in preference of either a 10% ethanol solution or water may exist. Once preference phenotypes were identified as either preferrers or avoiders of the ethanol solution, animals of a particular phenotype were sib mated in an attempt to intensify the trait. This study focuses on the avoider *P. leucopus* phenotype and presents data from several generations of sib mating.

Since an ADH^{o} phenotype exists in *P. maniculatus* and compromises ethanol metabolism, the possibility of a similar null phenotype in the *P. leucopus* avoider phenotype was evaluated using both PCR and starch gel electrophoresis. The *P. leucopus* avoider and preferrer specific liver ADH activity was also investigated to determine if differences may exist and correlate with any preference phenotype.

Initially, this study demonstrates that avoidance of 10% ethanol drinking solution seems to have a genetic basis in *P. leucopus.* We also suggest that the ADH^{o} phenotype is not present in the avoiders and preferrers we tested and is therefore not the underlying cause of avoidance in *P. leucopus.* The findings of the present study also indicate that differing genetic levels of ADH may exist and have an effect on ethanol preference in *Peromyscus.*

ETHANOL PREFERENCE IN PEROMYSCUS; AN ASSOCIATION WITH COMPROMISED ETHANOL METABOLISM

INTRODUCTION

Alcoholism is a human disease characterized by the excessive consumption of ethanol as well as the physical abnormalities which result from this behavior. It is generally accepted that genetic factors play a significant role in the development of alcoholism in humans (Cotton, 1979; Hhasbec and Omenn, 1981; Cloniger *et al.*, 1981; Cloniger, 1987). Studies often utilize two strategies to determine the relative contributions of genes and shared environment to the development of this disease. The first strategy, adoption studies, have demonstrated that sons of alcoholics adopted at birth have a four-fold higher risk for alcoholism than comparable controls (Goodwin *et al.*, 1973; Bohman, 1978). Another strategy, twin studies, focuses on concordance of monozygotic and dizygotic twins to determine likelihood of developing alcoholism. These studies consistently find that the rate of occurrence of alcoholism is significantly higher in monozygotic twins when compared to dizygotic twins (Kay, 1960; Loehlin, 1972). Because ethanol is a major causative factor of this disease, a full understanding of its biochemistry and pharmacology is essential to the elucidation of the mechanisms by which alcoholism may develop.

Studies on inbred strains of *Mus musculus* have also provided good evidence that alcohol preference and other alcohol-related behaviors have a strong genetic component. Inbred strains of mice are available which are known to differ in their preference for and tolerance of ethanol (Rodgers and McClearn, 1962). In these mice models, over ninety

percent of the varience in ethanol drinking behavior has been estimated to be due to genetic differences alone (Rodgers, 1966). Furthermore, Rodgers and McClearn (1962) found that when the C57BL/6 (preferring) and Balb/c (avoiding) strains were crossed, the F_1 generation was intermediate in ethanol preference. Rodgers and McClearn (1962) concluded that two loci, or two independent blocks of closely linked loci control the difference of ethanol preference between the two mouse strains. The genetic basis for these differences, however, has not been elucidated to date. A better understanding of ethanol metabolism in mammals may lead to an understanding of these genetic differences.

Ethanol is metabolized in the liver (Lieber, 1977) by three systems: alcohol dehydrogenase (ADH) (E.C. 1.1.1.1), catalase (E. C. 1.11.16), and the microsomal ethanol-oxidizing system (MEOS) (Rognstad and Grunnet, 1979). ADH, however, is responsible for catalyzing the oxidation of greater than 90% of the ethanol ingested in mammals tested to date (Vallee, 1985; Jornvall, 1994). The alcohol dehydrogenases are a zinc containing enzyme family which has been highly conserved among such diverse species as mammals, plants, and yeasts (Yokoyama and Harry, 1993). The enzyme is an oxidoreductase that oxidizes ethanol along with the coenzyme NAD^+ to acetaldehyde and NADH (Racker, 1949; Thurman *et al.*, 1974). The toxic acetaldehyde intermediate is converted to acetic acid via an aldehyde dehydrogenase (ALDH) and subsequently used in the Kreb cycle (Racker, 1949; Thurman *et al.*, 1974).

ADH isozymes in mammals, including humans, were originally grouped into class I, II, or III based upon electrophoretic, kinetic, and immunological properties (Strydom *et al.*, 1982)(Table 1). Human class I ADH is responsible for the metabolism of ethanol at

Table 1. Comparison of identified ADH enzyme systems between humans, *Mus musculus*, and *Peromyscus*.

		Human		*Mus musculus*		*Peromyscus*	
ADH Class	**Gene name**	**Subunit name**	**Number of Polymorphisms**	**Subunit name**	**Number of Polymorphisms**	**Subunit name**	**Number of Polymorphisms**
I	*Adh*$_1$	alpha	none	A_2	2	A_2	3
	Adh$_2$	beta	3				
	Adh$_3$	gamma	2				
II	*Adh*$_4$	pi	none	B_2	none	B_2	none
III	*Adh*$_5$	chi	none	C_2	2	C_2	none

physiological concentrations (Theorell, 1969). Class I isozymes include homo- and hetero-dimers which result from combinations of three different subunits (alpha, beta, and gamma), and are controlled by three non-allelic genes Adh_1 (alpha), Adh_2 (beta), and Adh_3 (gamma) (Yoshida, 1991). Interestingly, the Adh_1, Adh_2, and Adh_3 genes are tandomly organized on a 300 Kb fragment which suggests that they were derived by a gene multiplication of a single ancestral gene (Yasunami *et al.*, 1990). The beta and gamma loci are surprisingly polymorphic which further increases the multiplicity of this enzyme (Theorell, 1969). At least twenty different combinations of subunits have been identified to date in humans (Keung *et al.*, 1985). An interesting class I ADH beta polymorphism is common in Asian populations and exhibits a much higher catalytic activity (about a 100-fold) than that of the common allele (Yoshiba, 1991). This isoform may be partly responsible for the avoidance of ethanol by these individuals because acetaldehyde, the toxic intermediate, may be released into the bloodstream too quickly for the aldehyde dehydrogenase system to handle (Yoshiba, 1991). In addition to ethanol oxidation, these class I enzymes are also involved in metabolism of the neurotransmitters dopamine and norepinephrine, fatty acid metabolism, and steroid metabolism (Yoshiba, 1991).

The class II ADH enzyme is believed to be responsible for the metabolism of neurotransmitters as well as medium and long chain alcohols (Yoshiba, 1991). The class III enzymes are likely responsible for glutathione metabolism in addition to long chain alcohols (Yoshiba, 1991). No polymorphisms have been identified to date in either class II or class III and these enzymes are not responsible for ethanol metabolism at physiological concentrations (Yoshiba, 1991; Yokoyama and Harry, 1993).

The complexity of the human class I ADH enzyme has not been matched in rodent models. These animal models, however, have proven to be very useful in studying ADH ethanol metabolism and its genetics. In the house mouse (*Mus musculus*), these enzymes have been studied extensively (Algar *et al.*, 1983). Biochemical analysis of ADH in tissues of these mice indicate the presence of at least three different enzymes (designated A_2, B_2, and C_2) which are only present in the form of homo-dimers (Algar *et al.*, 1983). Genetic analysis using inbred mouse strains has provided evidence that these three homo-dimers are encoded by three separate genes (Holmes *et al.*, 1981). Furthermore, allelic variants have been described for the A_2 and C_2 loci (Holmes *et al.*, 1981). These variants have been used to map genes to a region on chromosome 3 which are only 0.8 centimorgans apart from one another (Holmes *et al.*, 1981). This has lead researchers to believe that these genes may also be the product of a recent gene duplication (Holmes *et al.*, 1981).

ADH A_2 and C_2 enzymes in mice have widely divergent catalytic properties (Algar *et al.*, 1983). The A_2 enzyme is similar to human class I ADH and is characterized by its high substrate specificity towards ethanol in the house mouse liver (Algar *et al.*, 1983). As in humans, it is the only ADH enzyme in this tissue that uses ethanol as a substrate at physiological concentrations, whereas B_2 and C_2 have a substrate specificity towards longer chain alcohols (Algar *et al.*, 1983). This system of enzymes seems to be conserved among rodents because identical systems have been identified in both rats (Koivisto and Eriksson, 1994) and hamsters (Keung, 1996).

Relative ADH activity has been investigated in several of these animal models. The C57BL strain of *Mus musculus*, which has been shown to prefer a 10% ethanol solution

when given a choice of this ethanol solution or water, has approximately twice the liver ADH activity of known avoider strains such as DBA and BALB (Koivisto and Eriksson, 1994; Rex *et al.*, 1984). Findings such as these suggest that ADH activity may have a genetic basis in *Mus musculus* because preference and liver ADH activity seem to be strain specific. Similarly, rats which prefer an ethanol solution have also been shown to have significantly higher ADH activity in liver extracts than those who avoid the same solution (Koivisto and Eriksson, 1994). Furthermore, the Syrian hamster or golden hamster (*Mesocricetus auratus*) always prefers a 10% ethanol solution when given a choice between this solution and water and their ADH activity is similar to that of the preferring house mouse strain C57BL (Keung, 1996; Balak *et al.*, 1982). Together, these findings indicate that ADH activity may be an underlying cause of preference or avoidance in some rodents.

The specific catalytic properties of purified ADH A_2 enzyme from C57BL and BALB inbred *Mus musculus* strains have been investigated. Rex *et al.* (1984) found that the K_m values for ethanol and NAD^+, the K_i for NADH, the pH optimum for ethanol oxidation, and the V_{max} for ethanol oxidation for the A_2 enzyme in these two strains are similar. Rex *et al.* (1984) also demonstrated that the electrophoretic and isoelectric focusing patterns as well as two-dimensional tryptic peptide maps of the purified enzyme from both strains are identical. Therefore, they concluded that the difference in ADH activity between the two strains cannot be due to catalytic properties of the enzyme. Zhang *et al.* (1987) later published the amino acid sequences of these strains showing that they are, indeed, identical.

Researchers also investigated the specific catalytic properties of ADH A_2 to determine if they may be the underlying cause of ADH activity differences between inbred strains, Wang and Singh (1984) were trying to determine if preference differences may be caused by differing magnitudes of ADH induction upon exposure to ethanol. By using different strains of mice, these researchers demonstrated that there is no direct evidence to suggest an association between ADH inducibility and preference because inducibility does not parallel this trait in many strains of mice. Wei and Singh (1988) also found that liver ADH activity is generally repressed in preferrers such as C57BL as well as avoiders such as DBA. Tagliabracci and Singh (1996), found that ethanol feeding is associated with alterations in the level of Adh_1-specific mRNA and that the alteration is genotype specific. Significant mRNA increases, however, were found in mice which demonstrate both the preferring and avoiding phenotypes supporting earlier findings that there is no likely no correlation between preference phenotype and inducibility.

Using *in vivo* radiolabeling and specific immunoprecipitating techniques, Balak *et al.* (1982) investigated genetic differences in the amount of ADH protein produced in the liver of these inbred strains of mice. Interestingly, they found that C57BL mice, which have approximately twice the level of liver activity as C3H mice, also have approximately twice the level of ADH protein synthesis in their liver. Using genetic crosses, Balak et al. (1982) also concluded that these differences are controlled by a single genetic locus. They proposed that this gene is regulated temporally by a locus which they named *Adh*-1-t. The location of the proposed *Adh*-1-t locus in relation to the structural gene is still unknown. Bond and Singh (1994) found that DNA sequence analysis yields no difference in known

5' transcriptional control elements between C57BL mice and BALB mice. Interestingly, Zhang *et al.* (1987) did find a difference in a purine pyrimidine repeat in the first intron of C57BL and BALB mice. This difference was a 101 bp deletion and may act as a cis acting control element (Zhang *et al.*, 1987).

Another mouse model which is used in the study of alcohol metabolism is *Peromyscus maniculatus* or the Prairie deer mouse (Cronholm *et al.*, 1992; Bradford *et al.*, 1992; Knecht *et al.*, 1993; Dudley and Winston, 1995). The deer mouse ADH A_2 system, which Zheng *et al.* (1993) found to be greater than 80% similar at the amino acid level to three class I human ADH isozymes was investigated by Burnett and Felder in 1978. They found two electrophoretic phenotypes of liver ADH A_2 in *Peromyscus maniculatus*. They were designated as the fast migrating liver ADH phenotype and the not detectable or null (ADH^o) phenotype. A third phenotype was found in *Peromyscus polionotus* and designated the slow migrating liver phenotype. By successfully mating these animals, Burnett and Felder (1978) demonstrated that the fast and slow phenotypes for ADH arise from protein products of two alleles at a single locus. Burnett and Felder (1978) also concluded that the third allele, ADH^o, either codes for a polypeptide incapable of forming an active enzyme or does not produce any protein product.

Zheng *et al.* (1993) later investigated the molecular basis of the ADH^o deer mouse to determine the underlying cause of the phenotype. They found that ADH A_2 mRNA is not detectable in the liver of the ADH^o deer mouse using ADH A_2 cDNA as a probe, indicating that no product is transcribed. Furthermore, Southern analysis using an essentially full-length ADH A_2 cDNA probe revealed no hybridization for the ADH A_2

gene indicating a deletion of the gene itself (Zheng *et al.*, 1993). These researchers concluded that the underlying cause of the ADH^{o} *P. maniculatus* phenotype is at least a partial deletion of the ADH A_2 gene.

Because of the lack of an active ADH A_2 protein in ADH^{o} deer mice, they have almost no liver ADH activity *in vitro* (Burnett and Felder, 1978). These findings are in agreement with other experiments which have indicated that ADH is responsible for approximately 90% of ethanol metabolism in mammals (Vallee, 1985; Jornvall, 1994). Interestingly, *in vivo*, ADH^{o} mice metabolize ethanol at approximately half the rate found for ADH-positive deer mice indicating that other systems may be contributing to ethanol metabolism in these animals (Burnett and Felder, 1980; Shigeta *et al.*, 1984). Indeed, the microsomal ethanol-oxidizing system is elevated about 2-fold in ADH^{o} deer mice (Burnett and Felder, 1980; Shigeta *et al.*, 1984). Contributions of other ethanol metabolizing pathways such as catalase have also been investigated with differing interpretations (Takagi *et al.*, 1986; Handler *et al.*, 1986; Alderman *et al.*, 1987; Norsten *et al.*, 1989). Both ADH^{o} and ADH-positive *Peromyscus maniculatus* as well as *Peromyscus leucopus* are the focus of this investigation.

In this study, outbred *P. leucopus* were initially subjected to an ethanol preference test to determine if differences in preference of either a 10% ethanol solution or water may exist. Once preference phenotypes were identified as either preferrers or avoiders of the ethanol solution, animals of a particular phenotype were sib mated in an attempt to intensify the trait. This study focuses on the avoider *P. leucopus* phenotype and presents data from several generations of sib mating. Since the ADH^{o} phenotype exists in *P.*

maniculatus and compromises ethanol metabolism (Burnett and Felder, 1978; Shigeta *et al.*, 1984), the possibility of a similar null phenotype in the *P. leucopus* avoider phenotype was evaluated. The *P. leucopus* avoider and preferrer specific liver ADH activity was also investigated to determine if differences may exist and correlate with any preference phenotype. It is hypothesized that compromised ethanol metabolism may be an underlying cause for the avoidance of an ethanol solution in both *P. leucopus* and *P. maniculatus.*

MATERIALS AND METHODS

Experimental Animals: Animals used in this study were from three sources. The White-footed deer mouse (*Peromyscus leucopus noveboracensis*) were obtained from an outbred colony at the College of William and Mary Population Lab. Prairie deer mice (*Peromyscus maniculatus bairdii*) were also obtained from an outbred colony at the College of William and Mary Population Lab. ADH^{o} (null) deer mice (*P. maniculatus)* were obtained from an inbred stock maintained at the Peromyscus Stock Center, University of South Carolina (Burnett and Felder,1978).

Ethanol Preference Test: The testing of *P. leucopus* began at a minimum of 60 days of age and continued for 10 consecutive days. All *P. maniculatus* were tested similarly at 90 $\pm$ 10 days old. ADH^{o} *P. maniculatus* were tested beginning at 213 days of age. During testing, animals were placed individually in opaque 20 cm X 40 cm X 18 cm plastic cages with wire tops and food (Harlan Teklad LM 485, Bartonville, Ill.) was available continuously. Two 100 ml test tubes fitted with a glass dropper protruding from the end allowing access to the fluid were positioned in an upright position and presented to the animals. One tube contained a 10% ethanol solution in tap water and the other contained plain tap water, each tube was filled with between 108 and 110 grams of the appropriate fluid and this initial weight was recorded to the nearest 0.01 gram along with the time of day, temperature, and humidity. At 24 hours $\pm$ 1 hour following the presentation of the

tubes the weight of each tube was again measured, recorded, and refilled with the appropriate amount of fluid. The position of the tube was switched daily. To correct for drip and evaporation, two cages without animals were set up on the same rack and treated in the same fashion as cages with animals. The water and ethanol measurements were averaged daily from two separate drip cages and these values were subtracted from the corresponding daily consumption value obtained to correct for drip and evaporation.

Consumption values were recorded to the nearest 0.01 gram daily, and net consumption was calculated by subtracting the corresponding average drip value for each fluid from day three to day ten. These values were then averaged for both the 10% ethanol corrected values and the water corrected consumption values. Next, these averaged daily values were added together in order to determine total consumption for each animal. The average corrected ethanol consumed was divided by total consumption in order to get a ethanol preference ratio (EPR). Those animals scoring a ratio of below 0.2 were designated as "avoiders".

Animal Care and the Establishment of Breeding Lines: Twenty *P. leucopus* were obtained at random from an outbred colony. They were subjected to the ethanol preference test and their scores were reported as a ratio of 10% ethanol solution consumed over water consumed instead of total consumption as above. These scores were then converted to the EPR by the following equation.

$$Y = \frac{X}{(X+1)}$$

Where X = the original value calculated
Y = conversion

These Y values were then used to determine the frequency distribution of EPR scores for the P_1 generation. The ranges used for the distribution of EPR scores were 0.00-0.20, 0.21-0.40, 0.41-0.60, 0.61-0.80, and 0.80-1.00. The animals in each category was then converted to a percentage by dividing the number by the total number of animals in the P_1 generation. The animals scoring between 0.00 and 0.20 were designated as avoiders and mated to establish the avoider lines. The ethanol preferrers (0.80 - 1.00) were also bred and will be the subject of another report.

The animals were kept either individually or with same sex sibling in a two-chambered, wire topped, opaque plastic enclosure, 12.8 X 27.8 X 14.5 cm on a side. Pine shavings approximately 2 to 3 cm deep, were used as bedding. Food (Harlan Teklad LM 485, Bartonville, Ill.) and tap water were available *ad lib*. Bedding was changed regularly and animals monitored daily.

When mated, animals were monitored regularly and pregnancies were noted. When litters were produced, the date was recorded and the wean date calculated and recorded. The young were weaned at twenty-one days of age and placed in the same cage type described above with their same-sex siblings. At this time, parents were recorded, toes were clipped corresponding to a number assigned to the animal, and cages were marked with animal numbers and date of birth.

Selection of Avoider Lines: The offspring of the P_1 pairs were tested and then maintained as described above. The frequency distributions of the avoider to preferrer phenotypes were determined using the EPR scores determined above. The distribution

ranges were the same as those used with the P_1 generation and the values are presented as percentages of the total number of animals in each generation.

Distribution of Ethanol Avoidance in Randomly Selected *Peromyscus maniculatus*: Sixteen *P. maniculatus* were selected at random from an outbred colony maintained at The College of William and Mary. Eight males and eight females were selected between 55 and 65 days old. These animals were subjected to the ethanol preference test and the EPR scores were used to determine frequency of distribution values within the same ranges as described previously.

Distributions of Ethanol Avoidance in ADH^o Animals: Four ADH^o animals were purchased from the Peromyscus Stock Center, University of South Carolina (Burnett and Felder, 1978). These animals were mated and six offspring were selected at between 55 and 65 days of age and subjected to the ethanol preference test. The distribution of ethanol avoidance was determined as a percentage of total ADH^o animals tested.

Tissue Extraction: Tissues of *P. leucopus* and *P. maniculatus* were treated identically. Animals were first weighed and then killed by cervical dislocation, followed by decapitation. The brain and liver were both removed within 2 min. The liver was placed in a preweighed 50 ml Falcon tube for final weighing. Four times the liver weight of extraction buffer (50 mM Tris-HCl pH 7.0, 1 mM dithiothreitol) (Zheng *et al.*, 1993) was added to the tissue to make a twenty percent tissue extract. The liver was homogenized within three minutes of removal with a Tissue Tearer on low speed for 30 seconds before placement on ice until all samples were collected. The homogenates were transferred to 30 ml Corex tubes and centrifuged for 20 minutes at 27,000 X g. Fifty ul of supernatant was

immediately added to the wells in the starch gel and the remainder was allotted into 100 ul portions in 1.5 ml Eppendorf tubes and stored at -80° C. Brain tissue was either used immediately or placed in cyrovials at -80° C until genomic DNA could be isolated.

Isolation of Genomic DNA: Initially, attempts were made to isolate DNA from tail snips in order to perform polymerase chain reaction on a large number of animals. These attempts were abandoned after polymerase chain reaction proved unsuccessful on these samples. An alternative approach used tissue from either frozen (-80C) or fresh brains that were homogenized in 10ml ice cold digestion buffer (100 mM NaCl, 10 mM Tris-Cl pH 7.4, 25 mM EDTA pH 8.0, 0.5% SDS, 0.1 mg/ml proteinase K) kept on ice. The homogenate was placed in 50 ml Falcon tubes and incubated at 50° C for 3 hours. After two phenol-chloroform (P/C) extractions the final aqueous layer was transferred to a clean 30 ml Corex tube. One tenth volume of 3 M sodium acetate was added along with 2 to 3 volumes ice cold ethanol to precipitate the DNA and spun at 5000 rpm for 5 min. at 4° C to pellet. The DNA was then resuspended in 600 ul TE (10 mM Tris-HCl, pH 7.0, 1 mM EDTA) and placed in a 1.5 ml Eppendorf tube and 2 ul RNase A was added and incubated at 37° C for thirty minutes. A P/C and subsequent chloroform extraction was performed on each tube in order to clean the extracted DNA before transfer to a new Eppendorf tube. The DNA was precipitated with one-tenth volume 3 M sodium acetate and 2 to 3 volumes ice-cold ethanol. After centrifugation, 100 ul TE was added to resuspend the DNA pellet. To evaluate the quality of the extraction and purification, 3 ul

of DNA was digested with restriction enzymes and compared with the uncut DNA on a 1.2% agarose gel.

Polymerase Chain Reaction: Degenerate primers were designed in an attempt to amplify the longest two exons in the ADH A_2 *P. leucopus* gene. To design these primers, mouse DNA (Ceci *et al.*, 1987), rat DNA (Crabb *et al.*, 1989), and *P. maniculatus* cDNA (Zheng *et al.*, 1993) were aligned and the most conserved 20 base pair lengths determined. The following degenerate forward and reverse primers from exon 5 and 6 were ordered from Genelink, Inc.

Primer sequences:

PLADHX5F - GGYACCAGCAGGTTCWCCTGC
PLADHX5R - GCGACTTKGACRGCAGAGCC
PLADHX6F - GTGACCCAAGGCTCCACMTG
PLADHX6R - GAAAAGTCCACCCCTCCRTC

Where Y = C,T; N = A,C,T,G; R = A,G; W = A,T; S = C,G.

Control Primers: The control primers for GAPD were designed by Frank Hurst (unpublished Honors Thesis) and ordered from Genelink, Inc. These primer sequences were:

PGADH10F - CAGGTKGTCTCCWSSGACTT
PGADH10R - TGTCATTGAGRGCAATKCCA

Where Y = C,T; N = A,C,T,G; R = A,G; W = A,T; S = C,G.

All primers were diluted to 10^{-1} and 10^{-2} and run out on a 1.2% agarose gel to determine relative concentrations. All primers were used at the 10^{-2} concentration, except for the 10^{-1} dilution, which was most suitable for PLADHX6F. Moderately stringent temperatures were used with PCR in a Perkins Elmer Gene Amp 2400 PCR machine in order to obtain the specific target products: 95° C for 5 min; 40 cycles of (94° C for 1 min for denaturation and 55° C for 2 min for annealing followed by 72° C for 3 min for elongation); followed by 72° C for 7 min and a hold at 4° C. In each reaction tube, 1 ul of DNA and 5 ul each of the appropriate primer was used. All reaction products were run out on 1.2% agarose gels.

Exon 5 and Exon 6: PCR for exon 5 was performed using 5 ul of the 10^{-2} solutions of both the exon 5 forward and exon 5 reverse primer dilutions along with 1 ul of *P. leucopus* genomic DNA. PCR for exon 6 was performed using 5 ul of the 10^{-2} exon 6 forward primer solution and 5 ul of the 10^{-1} exon 6 reverse primer solution along with 1 ul of genomic DNA form *P. leucopus*. A 200 base pair fragment was obtained from the exon 5 reaction and a 220 base pair fragment was obtained from the exon 6 reaction. Each reaction mixture was run as described previously, and the product was evaluated on a 1.2 percent agarose gel. The exon 5 band was Genecleaned according to the manufacturer's instructions (Geneclean II Kit BIO 101, Inc.). The Genecleaned exon 5 product and the PCR exon 6 product were cloned into plasmid vectors using TA cloning Kit (Invitrogen) according to the manufacturer's instructions. Several light blue colonies were chosen and

the plasmid DNA was isolated. The product was digested with the restriction enzyme EcoR1 and run on a 1.2% agarose minigel.

Sequencing of Exon 5 and Exon 6: Cloned fragments were sequenced using the dideoxy termination method (Sanger *et al.*, 1977). A sequencing kit (Sequenase 2.0, United States Biochemical) was used according to the manufacturer's instructions. The sequencing reaction was run on a 6% acrylamide gel (46 g urea, 15 ml 40% acrylamide, 5 ml 10xTBE buffer, 18 ul TEMED, 700 ul 10% APS) which had polymerized for three hours. The gel was pre-run at 1550 V for 30 minutes before loading. The wells were rinsed before loading with 0.5xTBE (Tris-Borate EDTA) buffer to remove excess urea. Three ul of each sequencing reaction was loaded onto the gel after boiling for 3 minutes. The gel was run at 2000V for 2.5 hours. The clamps were then removed from the sequencing apparatus and the top plate was carefully removed. Filter paper (3MM) was pressed onto the gel and the gel was subsequently lifted off of the bottom plate and covered with saran wrap. The gel was dried in a Bio-Rad gel dryer for 4 hours and then exposed to Xray film (Fuji) for four days before being developed and read for base sequencing.

PCR to Determine the Presence of the ADH A_2 Gene: Exon 5 primers were used to indicate the likely presence or absence of the ADH A_2 gene in 6 *P. leucopus* which had EPR scores above 0.80 (preferrers) and 6 *P. leucopus* which had EPR scores below 0.15 (avoiders). The ADH^o *P. maniculatus* was used as a negative control. As a positive

control in all tubes, the GAPD control primers (PADH10F and PADH10R) were used. Each reaction mixture was run as described previously and the product was evaluated on 1.2% agarose gels.

Starch Gel Electrophoresis to Determine the Presence or Absence of ADH A_2 Enzyme: At 4° C, 50 ul of fresh 20% liver tissue extracts were loaded into 100 ul wells of a 12% starch gel (Zheng *et al.*, 1993). The starch gel was prepared with 450 ml Tris/ Citric Acid Buffer (8 mM Tris and 3 mM Citric Acid adjusted to a pH of 7.2 with NaOH) and 52 grams of Potato Starch (Sigma, S4501). The solution of starch and gel buffer were then heated until a phase change was noted, degassed with water pump aspirator until small bubbles were removed, and poured immediately into a previously leveled 19 cm X 23 cm gel mold ~0.8 cm thick. A 0.1 X 1.0 cm comb was used to form the well. The gel was allowed to set up for 45 minutes at room temperature and transferred to 4° C for at least 3 hours before use. The gel was connected to buffer chambers containing a buffer 27.5 times more concentrated than the buffer used to make the gel. Next, 3MM paper cut to the width of the gel was used to contact the buffer in the chamber to the gel. Tissue extracts were loaded into wells within 30 minutes of collection and allowed to run at 250 volts for 30 minutes and then at 142 volts (7V/cm) for 14 hours at 4° C. Four gels were run for this study and all extracts were repeated at least once.

Preparation of a Standard Curve and Control : Control liver tissue was extracted and starch gel was prepared as above (Zheng *et al.*, 1993). Fifteen aliquots of the control liver

extract were prepared and frozen immediately to be used as a standard on each starch gel above. Next, a standard curve was prepared as follows: 20 ul tissue extract in 80 ul distilled water as a 20% solution, 40 ul tissue extract in 60 ul distilled water as a 40% solution, 60 ul tissue extract in 40 ul distilled water as a 60% solution, 80 ul tissue extract in 20 ul distilled water as a 80% solution, and 100 ul tissue extract as the 100% solution. An additional 100% aliquot (100 ul) was placed at -80° C for 20 minutes, thawed, and loaded on the same gel as the standard curve. Gels were run at 250 volts for 30 minutes and then at 142 volts (7V/cm) for 14 hours at 4° C.

Starch Gel Electrophoresis to Determine Relative ADH Activity: Fresh tissue extracts were aliquoted into five 100 ul sterile 1.5 ml Eppendorf tubes with the remainder of the extract placed in a 20 ml Falcon tube to be stored at -80° C. Liver extracts of six prefers and six avoiders were taken from -80° C storage and allowed to thaw on ice. Once thawed, the extracts were taken to a 4° C cold room where a 30 ul aliquot of each liver extract was loaded into 50 ul wells on a 12% starch gel prepared as described above (Zheng *et al.*, 1993). The gels were allowed to run at 250 volts for 30 minutes and then at 142 volts (7V/cm) for 14 hours at 4° C.

Starch Gel Electrophoresis to Determine Isozymes Present: Tissue extracts were prepared as described above except that the tissues, once thawed, were taken to the 4° C cold room and a 3 mm x 6 mm piece of 3MM paper was allowed to absorb each tissue extract until saturated. These absorbed samples were loaded as follows. The starch gel

was sliced across mid-line approximately 8 mm deep, separated, and the tabs of 3MM paper were placed in the slice. The gels were run at 250 volts for 30 minutes and then at 142 volts (7V/cm) for 14 hours at 4° C.

Staining: After the gels were run, they were sliced 2 mm thick at 4° C with a horizontal gel slicer. A 0.7 percent agar overlay solution was prepared just before slicing using 100 mM Tris-HCl (pH 8.0), 250 m*M* ethanol, 10 mM Pyruvate, 0.9 mM MTT, 0.3 mM PMS, and 0.38 mM NAD (Holmes *et al.*, 1981). Fifty ml of this solution was poured evenly onto the gel surface and the gel was then incubated for 20 min at 37° C.

Analyzing the Gel: After 20 minutes of incubation, the gel was photographed. The NIH image analysis software (Scion Image for the PC) was used to analyze the photographs after digital scanning (Vivitar,Inc.). The average density of each band was determined by averaging all pixels within a determined area. The size of this area was kept constant for a particular gel and a background gel value was determined above each band. The background was subtracted from the band value in order to control for varying gel density. The value for each lane was then divided by the control and a ratio determined. A Students t-test was used to determine if the values for the prefers and avoiders were statistically significantly different.

Activity Analysis Assay: All tissue extractions were stored at -80° C until assays were performed. ADH activity was assayed at 27° C in 0.1 M glycine buffer (pH 10.7)

containing 1.7 mM NAD and 15 mM ethanol as substrate. NADH production was measured at 340 nm using a Boush and Lomb Spectronic 2000 spectrophotometer. All reactants and enzyme extract were added to cuvette initially and blank absorbence was taken. Next, ethanol was added to initiate the reaction and recordings were taken every fifteen seconds for four consecutive minutes.

Preparation of NADH Concentration vs. Absorbency Standard Curve: To prepare a standard curve for NADH concentration vs. absorbence, 10 uM, 25 uM, 50 uM, 250 uM, and 1 mM NADH solutions were prepared in 0.1 M glycine buffer at a pH of 10.7. Absorbencies of these concentrations were taken at 340 nm and a curve was plotted. The slope of this curve was determined to be 0.00533 and was used to convert values from units of absorbence to uM amounts of NADH using the following equation.

$$\frac{X}{0.00533} = Y$$

Where X = unit of absorbence
Y = uM amount of NADH

Analysis of Preferrer, Avoider, and ADH° Liver Extracts

All samples were initially extracted, homogenized as a 1:4 W/V ratio of tissue to buffer, aliquoted, and placed in the -80° C freezer. These samples were analyzed for the rate of NADH production measured at 340 nm for six *P. leucopus* preferrers, eight *P. leucopus* avoiders, and two ADH° *P. maniculatus* mice. Values are expressed in micromolar amounts of NADH produced per minute per 20 ul of liver extract.

To determine the possible effect of thaw time on sample reaction rates, a test was performed in which 7 aliquots of the same liver extract were removed from the -80° C freezer at the same time and allowed to thaw on ice. The thaw times were recorded for each sample when the activity analysis assay was performed.

Eight *P. leucopus* were selected randomly from The College of William and Mary Population Lab outbred stock and subjected to the activity analysis assay. These animals were all approximately 13 months old. Also, two inbred strains of *Mus musculus* were selected and subjected to the ethanol preference test, initially and three weeks later were sacrificed and activity analysis was performed on 2 individuals of each strain.

All work involving animal subjects was approved by the Institutional Animal Care and Use Committee, College of William and Mary. All work involving infectious agents and or recombinant DNA has been approved by the Institutional Biosafety Committee, College of William and Mary. All work involving radioactive materials was approved by the Radiation Safety Officer, Department of Biology, College of William and Mary.

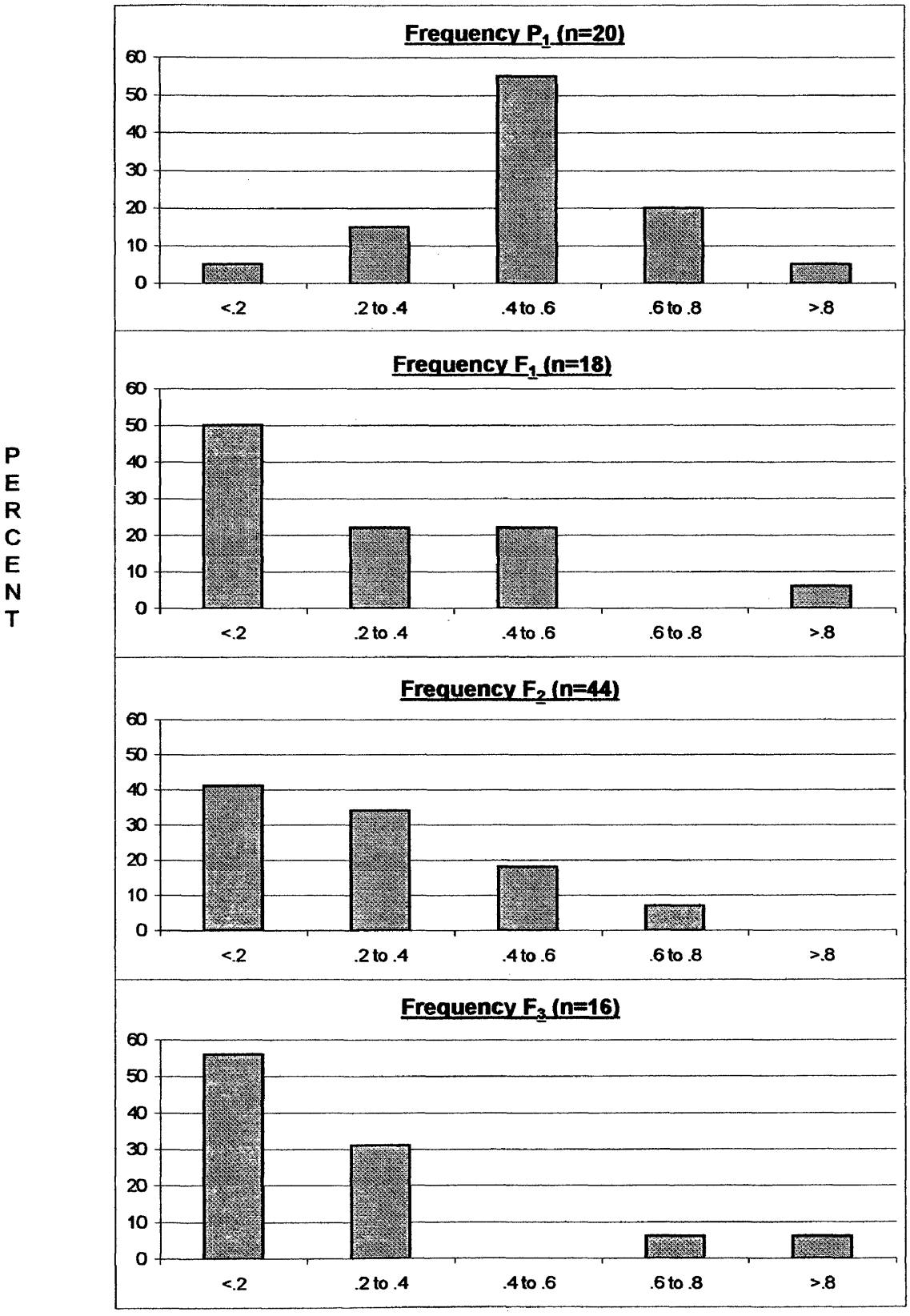
RESULTS

I. Identifying Ethanol Preference Traits in *P. leucopus*

Initial Testing of Randomly Selected Animals: Twenty *P. leucopus* were selected at random from an outbred stock and designated the P_1 generation. They were subjected to the ethanol preference test and their EPR scores were used to determine frequency of distributions (Fig. 1). The majority of the animals scored were intermediate between 0.2 and 0.8 (columns 2, 3, and 4) and did not strongly prefer or avoid the ethanol solution. Approximately five percent of the animals tested scored below 0.2 and were termed "avoiders" (column 1). The animals that scored above 0.8 also represent approximately five percent of the population and were termed "preferrers" (column 5). These results indicate a near normal distribution curve for the twenty randomly selected *P. leucopus* in the parental (P_1) generation.

Breeding Experiments for the Avoider Trait: Offspring from initial pairings of the P_1 generation and from 2 succeeding generations (F_1, F_2, and F_3 respectively) were also subjected to the ethanol preference test between 55 and 65 days of age and the lowest EPR scorers were sib mated in an attempt to intensify the avoidance trait (see Appendix 1 for pedigrees). The frequency distributions of ethanol preference for these generations were determined using EPR scores described earlier (Fig. 1). From the P_1 generation to the F_1 generation, there is an increase in the percentage of avoiders in the population from

Figure 1. Frequency distribution of ethanol preference scores for the P_1, F_1, F_2, and F_3 generations. Values are expressed as percentage of total animals tested in each generation.

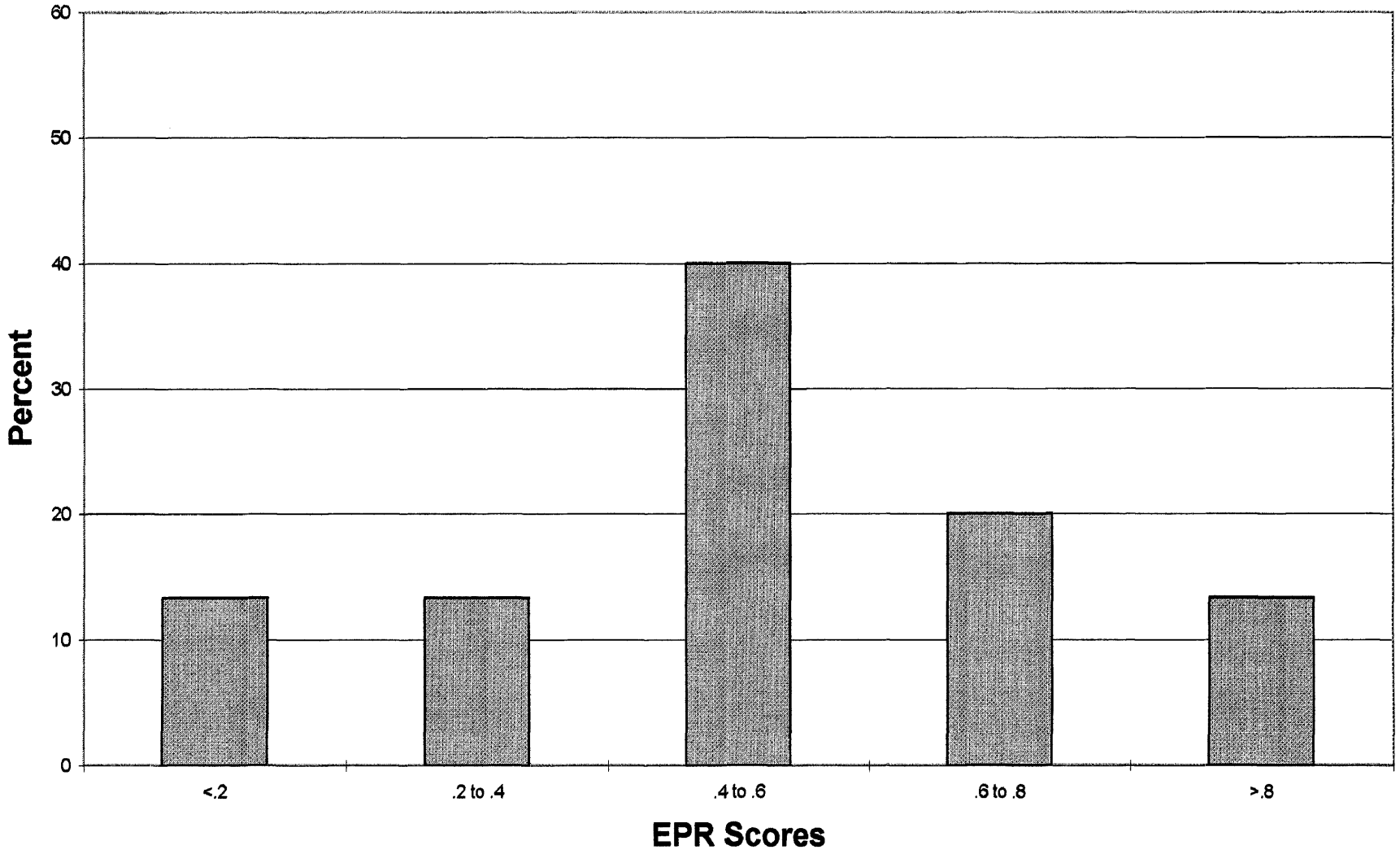

Frequency P1 (n=20)
Frequency F1 (n=18)
Frequency F2 (n=44)
Frequency F3 (n=16)
60
50
40
30
20
10
0
<.2
.2 to .4
.4 to .6
.6 to .8
>.8
PERCENT
SCORE

5% to 50%. There was also a slight increase in the number of animals scoring between 0.2 and 0.4. These trends continue through the F_2 and F_3 generations along with a gradual decrease in the number of animals scoring intermediately between 0.4 and 0.6. A Two Sample t-Test (unpooled) was used for statistical analysis between generations because the curves of the populations differed. The F_1 generation EPR scores are significantly lower ($P = 0.003$) than P_1 generation scores. There are no significant differences ($P = 0.43$) between the EPR scores of the F_1 and F_2 generations. There are also no significant differences ($P = 0.51$) when the F_2 and F_3 generation EPR scores are compared. Interestingly, the frequency of preferrers in each successive generation did not decrease.

II. Ethanol Preference Distributions in *P. maniculatus*

Preference Distribution of Randomly Selected Animals: Fifteen random *P. maniculatus* were selected from an outbred colony and the frequency distribution of ethanol preference distributions for these animals were determined using scores from the ethanol preference test described earlier (EPR) (Fig. 2). The normal distribution curve for the randomly selected *P. maniculatus* appears to be similar to the curve obtained for the randomly selected *P. leucopus* P_1 generation (Fig. 1). The majority of the animals scored intermediately between 0.2 and 0.6. The number of preferrers and avoiders were equal at approximately 13 percent. The EPR scores are not significantly different ($P = 0.87$) when comparing the twenty *P. leucopus* tested initially (P_1 generation) and the fifteen *P. maniculatus* selected at random and tested. These data indicate that *P. maniculatus* have a near normal distribution similar to *P. leucopus.*

Figure 2. Frequency distribution of EPR scores for random selected *P. maniculatus* (n=16).


60
50
40
30
20
10
0
Percent
<.2
.2 to .4
.4 to .6
.6 to .8
>.8
EPR Scores

Distributions of Ethanol Preference in ADH^{o} Animals: Six ADH^{o} *P. maniculatus* were subjected to the ethanol preference test. The mice scored 0.01, 0.10, 0.06, 0.11, 0.16, and 0.05 and are considered avoiders. Since each of these mice have EPR scores below 0.20, they are all considered to be avoiders. These scores are significantly lower ($P < .001$) than the scores of 15 randomly selected *P. maniculatus* tested. These numbers were also significantly lower ($P = 0.018$) than the *P. leucopus* F_1 generation.

III. Analysis of Exon 5 and 6 *P. leucopus* ADH Genes

Polymerase Chain Reaction: Polymerase chain reaction was performed at medium stringency on *P. leucopus* genomic DNA. Exon 5 primers amplified approximately a 200 bp fragment and exon 6 primers amplified approximately a 230 bp fragment. The sequence of these fragments is presented in Fig. 3. Fragment sequences were compared with the Genebank Database and the 15 best matches are presented in Appendix 2. Sequences for *P. leucopus* exon 5 and exon 6 are approximately 98% identical to the ADH A_2 gene in *P. maniculatus* (Zheng *et al.*, 1993). All other matches were also to ADH sequences from various mammals including humans (Appendix 2).

Polymerase Chain Reaction to Determine Presence of ADH Gene: *P. leucopus* preferrer and avoider DNA extractions yielded both the control GAPD band and the ADH exon 5 band indicating the likely presence of the ADH gene (Fig. 4). PCR reactions not showing the control GAPD band were discarded from consideration. The base pair length of the exon 5 band on the agarose gel which was cloned and sequenced earlier was

Figure 3. Partial sequences of *P. leucopus* ADH exon 5 and 6.

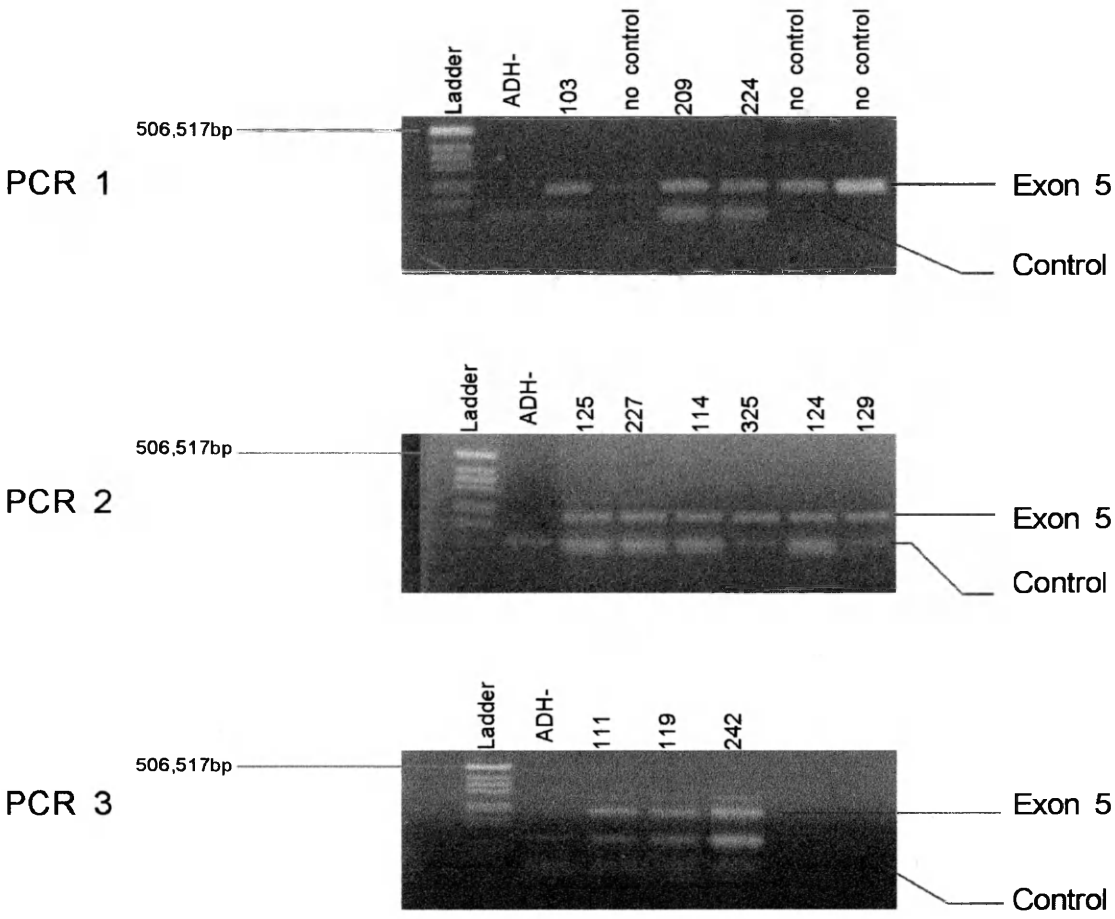
Sequence of Exon 5:

CCAGCAGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCA
TCAGCACCAGCACTTTCTCCCAGTCACTGTGGTAGATGAGAT
GGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAG
TCTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTG
CTG TCAAAGTCGC

Sequence of Exon 6:

GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGT
GTTGGTCTGTCTGTGATCATTGGCTGTAAAACAGCAGGAGCG
GCCAGGATCATCGCTGTGGACATCAACAAAGACAAGTTTGC
AAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTC
TAGACTATAGCAAGCCCATCCAGGAAGTACTCCAGGAA

Figure 4. PCR experiments to demonstrate presence or absence of exon 5. The number above each lane represents the corresponding animals DNA sample used in each reaction. Reactions with no control bands were disregarded.


PCR 1
506,517bp
Ladder
ADH-
103
no control
209
224
no control
no control
Exon 5
Control
PCR 2
506,517bp
Ladder
ADH-
125
227
114
325
124
129
Exon 5
Control
PCR 3
506,517bp
Ladder
ADH-
111
119
242
Exon 5
Control

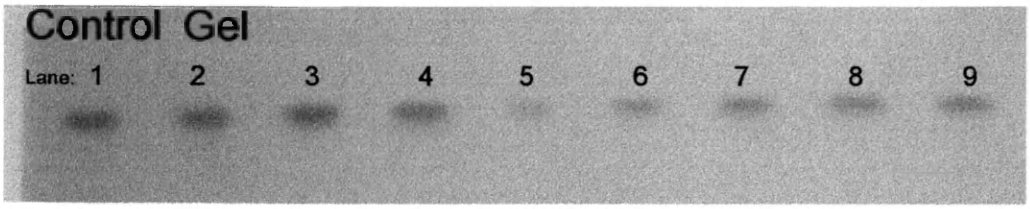
compared to the exon 5 bands which resulted from these experiments. All bands appear to be the same base pair length indicating that they are likely to be exon 5. Both ADH^{o} mice DNA extracts yielded only the GAPD control band.

III. Starch Gel Electrophoresis

Preparation of Standard Curve: Fresh dilutions of 20%, 40%, 60%, 80%, and 100% liver extracts were prepared from aliquots of the control liver extract to demonstrate concentration effects on staining intensity (Fig. 5). NIH image analysis was then performed on photographs taken of the gel after twenty minutes of staining. The density values of the dilutions were plotted and the resulting curve indicates a semiquantitative relationship between pixel darkness and concentration of liver extracts. According to the analysis program, the 100 percent dilution is approximately three times as dense as the 20 percent dilution (Fig. 5). On the same gel, repeats of fresh samples were included in extra lanes to get an idea of variability. The pixel density of the frozen sample in lane one is similar to the 100 percent repeats indicating that freezing may not have any adverse affects on ADH activity.

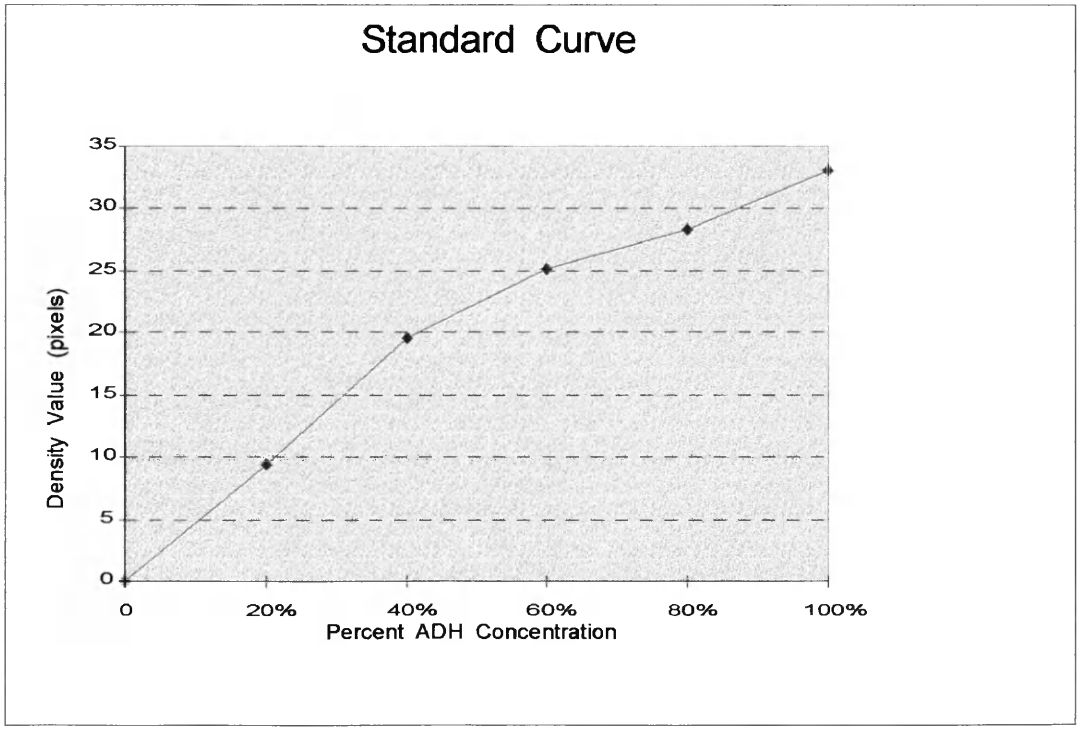
Electrophoresis to Determine Presence of the ADH A_2 Enzyme: Fresh liver extracts from 6 *P. leucopus* preferrers, 6 *P. leucopus* avoiders, and 2 ADH^{o} *P. maniculatus* were run on 12 percent starch gels. As expected, no bands were detected in the ADH^{o} *P. maniculatus* liver extracts used in each gel. Control aliquots prepared earlier were thawed on ice and loaded on each gel ran. The resulting control bands appeared similar in each

Figure 5. Gel demonstrating concentration effects on activity staining, effects of repeating the same sample, and the effect of snap freezing a sample. The percent extract equals the concentration of the extract loaded onto the gel. The standard curve was then plotted for lanes 5 through 8 as well as the average of all 100% extracts.



NIH Image Analysis:

Lane	Extract	Area (pixels)	Band Density (uncorrected)	Gel Background	Band Density
1	Frozen	1176	92.82	123.7	30.88
2	100%	1176	106.45	137.36	30.91
3	100%	1176	112.83	150.98	38.15
4	100%	1176	128.45	164.83	36.38
5	20%	1176	165.69	175.06	9.37
6	40%	1176	160.01	179.51	19.5
7	60%	1176	155.85	181.06	25.21
8	80%	1176	153.88	182.25	28.37
9	100%	1176	148.67	176.43	27.76

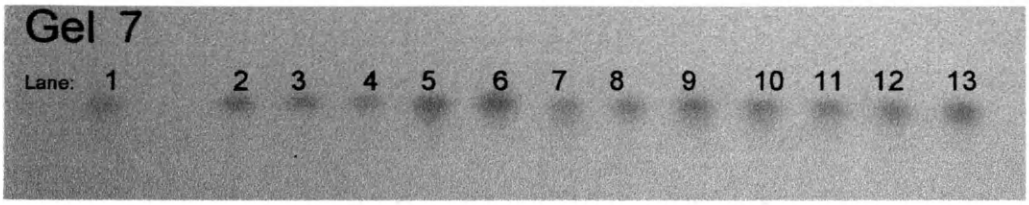


gel. Anodally migrating ADH A_2 enzyme activity was detected in all *P. leucopus* preferrer and avoider extracts. These results indicate all *P. leucopus* tested have the ADH A_2 enzyme (Appendix 3). Since each gel varies in density no attempts were made to compare densities of bands between different gels.

NIH Image Analysis on gel 7: As a semiquantative method to indicate if differing levels of ADH activity may be present in these animals, NIH image analysis was used to analyze gel 7 where all extracts were run at once (Fig. 6). The highest pixel density value in arbitrary units is 44.82 and belongs to animal 209 which is preferrer. The lowest value is 18.26 and belongs to animal 325 which is an avoider. Furthermore, the average pixel density value of 24.13 ± 5.27 for the avoiders is significantly lower ($P = 0.002$) than the average preferrer value of 37.24 ± 5.52. These results indicate that the avoider liver extracts have significantly less ADH activity than the preferrer liver extracts.

Comparison of Isozyme Types in *P. leucopus* Avoiders and Preferrers: Liver extracts from the available six *P. leucopus* preferrers and six *P. leucopus* avoiders were run out on a 12% starch gel to determine isozyme type present (Fig. 7). Three avoiders and one preferrer (animal numbers 224, 227, 325, and 209) express a single ADH A_2 band which migrates fast. The remaining 3 avoiders and 5 preferrers express two ADH A_2 bands which migrate intermediately and slow. There appears to be no correlation between these isozyme types and ethanol preference in *P. leucopus*. Additionally, when comparing only

Figure 6. Analysis of Gel 7. Extracts from both preferrers and avoiders were run at once and photographed. Values are expressed as average pixel darkness. Mean values were then calculated and presented with the standard deviation.

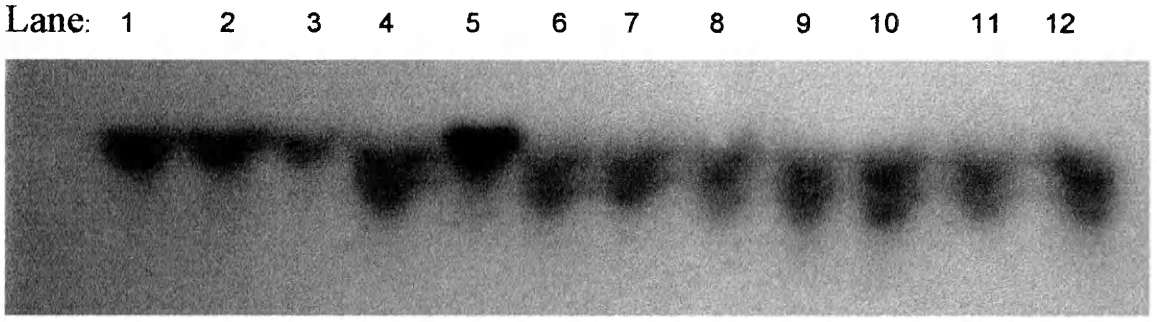


NIH Image Analysis:

Lane	Animal	Type	Area (pixels)	Band Density (uncorrected)	Gel Density	Band Density
1	control		756	113.78	129.17	15.39
2	227	A	756	123.19	145.29	22.1
3	224	A	756	132.68	153.91	21.23
4	325	A	756	140.05	158.31	18.26
5	103	P	756	124.15	162.76	38.61
6	209	P	756	125.1	169.92	44.82
7	124	A	756	152.5	176.26	23.76
8	125	A	756	155.31	181.19	25.88
9	129	P	756	147.89	185.05	37.16
10	242	A	756	153.47	187.01	33.54
11	114	P	756	158.01	187.88	29.87
12	111	P	756	156.87	189.01	32.14
13	119	P	756	145.74	186.56	40.82

Avoider		Preferrer	
Mean	S.D.	Mean	S.D.
24.13	5.27	37.24	5.52

Figure 7. Gel demonstrating the isozyme banding pattern of the indicated animals. A = avoider and P = preferrer



Lane	Animal	Preference
1	224	A
2	227	A
3	325	A
4	103	P
5	209	P
6	125	A
7	124	A
8	129	P
9	242	A
10	114	P
11	111	P
12	119	P

the fast migrating bands, the band from animal 209 appears approximately twice as dark as the bands for 224, 227, and 325.

IV. ADH Activity Analysis

Standard Curve of NADH Concentration vs. Absorbency: The absorbencies of different concentrations of NADH were taken at 340 nm and a standard curve was plotted. The slope of this curve was determined to be 0.00533 and is used to algebraically convert values of absorbency to uM amounts of NADH/min/20 ul liver extract. There is a linear relationship between the amount of NADH present and absorbency.

Analysis of *P. leucopus* Preferrer and Avoider as well as *P. maniculatus* ADH^{o} Liver Extracts: Values for all animals are expressed as uM NADH/min/20 ul liver extract (Table 2)(Appendix 4). The highest liver ADH activity value is 28.52 and belongs to animal 209 which is a preferrer. The lowest value is 10.88 and belongs to animal 325 which is an avoider. Furthermore, the mean preferrer ADH activity for 6 animals was found to be 21.3 $\pm$4.5. The mean for 8 avoiders animals was found to be 15.8 $\pm$3.0. These results indicate that the average ADH activity of the avoider extracts are significantly (P = 0.032) lower than the preferrer liver extracts using the Two Sample t-Test (unpooled). The ADH^{o} mice, on the other hand, had almost no liver ADH activity. Two mice (numbers 4 and 5) out of the 8 *P. leucopus* selected at random had abnormal livers. One liver was almost twice the normal size and spotted (number 4) and the other liver (number 5) was very small, dark, and irregular shaped. The data from these two animals (values x_4 and x_5) were not

Table 2. The mean $\pm$ SD of the NADH activity expressed as uM NADH/ min/ 20 ul extract for *Peromyscus leucopus* avoiders, preferrers, and outbred random; *Peromyscus maniculatus* ADH^{o}; and *Mus musculus* DBA and C57BL.

Species	Animals	Mean ADH Activity (uM NADH/min/20 ul extract)	Standard Deviation
P. leucopus	Preferrers n=6	21.3	4.5
P. leucopus	Avoiders n=8	15.8	3.0
P. leucopus	Random n=6	19.0	7.7
P. maniculatus	ADH^{o} n=1	1.3	
Mus musculus	DBA n=2	19.9	-
Mus musculus	C57BL n=2	30.8	-

included in further analysis. The mean ADH activity value for the 6 remaining *P. leucopus* animals selected at random was intermediate (19.8 $\pm$ 7.4) between the preferrers and avoiders. This sample with a much larger standard deviation suggests a greater variation within this random collection of outbred animals. There are no significant differences between the average ADH activity of avoiders and preferrers when compared with the random sample (P = 0.27 and 0.67, respectively). When comparing the male avoiders with the female avoiders, no significant (P = 0.55) differences were found.

Using Inbred Strains of *Mus musculus* to Validate Tests: One male and female from each of the C57BL and DBA house mouse strains were obtained from Charles River Laboratories and used to validate the ethanol preference test and the ADH activity test. The individual male and female C57BL mice scored, 0.81 and 0.67 respectively on the ethanol preference test and therefore resemble scores obtained by Mclearn and Rodgers (1959). The individual male and female DBA mice scored 0.00 and 0.14 respectively and also resemble ethanol preference scores obtained by Mclearn and Rodgers (1959). The DBA mice had approximately 65% of the liver ADH activity as the C57BL mice (Table 1). Taken together these data indicate that the ethanol preference test and the ADH activity analysis used in this study are reliable and consistent with other published reports.

DISCUSSION

Initially, this study demonstrates that avoidance of an ethanol solution seems to have a genetic basis in *P. leucopus.* Twenty *P. leucopus* were randomly selected and subjected to an ethanol preference test. The lowest range of scores (0.00 to 0.20) were defined as "avoiders" and the highest range of scores (0.81 to 1.0) were defined as "preferrers". The majority of the animals scored intermediately between 0.2 and 0.8 appearing to neither prefer nor avoid the ethanol solution. Approximately five percent of the animals tested scored below 0.2 and are considered avoiders. Similarly, approximately five percent of the animals scored above 0.8 and are considered preferrers. These results suggest that there may be a normal distribution of ethanol preference in a wild population of *P. leucopus.*

To study the avoider phenotype representations of this group, the avoiders were sib mated and the frequency distribution of ethanol preference for each subsequent generation was determined. From the P_1 generation to the F_1 generation, there was a significant ($P = 0.003$) decrease in scores resulting in a increase in the percentage of avoiders in the population from 5% to 50%. The results from the F_1 generation indicate a response to selection and therefore we may conclude that the avoidance trait has a genetic basis in *Peromyscus leucopus.* The importance of genetic factors in alcohol preference in *Mus musculus* has also been demonstrated (Rodgers, 1966). Genetic variance was attributed to

97% of drinking behavior, with other factors such as sex differences and litter size attributing to only 3% of the total variance (Rodgers and Thiessen, 1964).

In the two subsequent generations (F_2 and F_3), the percentages of the avoider phenotype were 41 and 56 respectively. The percentages of avoiders did not significantly increase after the initial F_1 generation but the numbers of animals scoring in the intermediate phenotype (.40 to .60) was sharply reduced. The persistence of a low number of preferrers in the F_1 and F_3 generations (6% in both cases) suggest that the preference trait may be encoded by multiple genetic loci. Mendelian studies with crosses of inbred *Mus musculus* strains known to prefer and avoid ethanol have indeed demonstrated that the ethanol preference phenotype in the house mouse has a genetic basis and is encoded by two loci or two independent blocks of closely linked loci (Fuller, 1964; Tagliabracci and Singh, 1996).

Two inbred strains of *Mus musculus* with known phenotypes were used to compare the ethanol preference test used in this study with other published results. The C57BL strain had been previously shown to be preferrers scoring approximately 0.85 on preference tests (Rodgers and McClearn, 1962). The DBA strain tested by Rodgers and McClearn (1962) were shown to be avoiders and score approximately 0.05 on ethanol preference tests. The male and female C57BL mice tested in this study scored 0.67 and 0.81, respectively, and the male and female DBA mice scored 0.014 and 0.005, respectively. These results demonstrate that the ethanol preference test scores reported here are reliable and consistent with other studies.

The frequency distribution of ethanol preference in fifteen randomly selected *Peromyscus maniculatus* from an outbred population was also investigated. We found this population to have a frequency distribution similar to the twenty randomly selected *P. leucopus* we tested earlier (P_1 generation). Forty percent of these animals scored in the intermediate range between 0.4 and 0.6. The other phenotypes were approximately equally distributed in each of the other four ranges (Fig. 2).

Additionally, 6 ADH^o *P. maniculatus* were tested because these mice are known, *in vivo*, to metabolize ethanol at approximately fifty percent the rate of ADH-positive mice (Burnett and Felder, 1980; Shigeta *et al.*, 1984). These animals all avoided the ethanol solution and had an mean EPR score of 0.08 ± 0.05 which is significantly lower than the random selected *P. maniculatus* EPR scores mentioned above. By comparing preferences between outbred *P. maniculatus* and the ADH^o mouse, we hypothesized that compromised ethanol metabolism may be at least one underlying cause of ethanol avoidance seen in these mice.

To investigate whether *P. leucopus* avoiders may also lack the ADH A_2 enzyme, two experiments were undertaken. Initially, PCR primers were designed for *P. leucopus* Exon 5 and 6 using *P. maniculatus* ADH A_2 cDNA sequence (Zheng *et al.*, 1993). Using genomic DNA isolated from three randomly selected P. leucopus, gene fragments were amplified, cloned and sequenced. The sequences of these exons are approximately 97% identical over the entire length of exon 5 and 6 combined when compared to those of *P. maniculatus* (Zheng *et al.*, 1993). This high degree of similarity suggests that the ADH A_2 enzyme responsible for ethanol metabolism may be nearly identical in these two species.

This similarity was expected since there is a 94% similarity between *P. maniculatus* and *Mus musculus* ADH A_2 amino acid sequences (Zheng *et al.*, 1993). These sequences are also most similar (86%) to the human class I ADH beta isozymes. In humans, the class I beta enzyme is polymorphic. Interestingly, one polymorphism has been discovered in Asians who have a higher likelihood of avoiding ethanol due to an increase in enzyme activity when compared to the common enzyme (Shibuya *et al.*, 1989).

PCR performed using primers for exon 5 on total genomic DNA isolated from six different *P. leucopus* avoiders and preferrers demonstrates the likely presence of the ADH A_2 gene in these animals. In each reaction where the control GAPD band appeared in *P. leucopus* preferrer and avoider reactions, the exon 5 primers amplified a fragment similar to the one sequenced earlier. Furthermore, ADH^o *P. maniculatus* genomic DNA reactions yielded no band for exon 5 when the GAPD control band was present. The absence of an exon 5 band in ADH^o *P. maniculatus* is consistent with Northern and Southern blot experiments performed by Zheng *et al.* (1993) which found that there must be a deletion of the ADH A_2 gene in these mice.

The presence or absence of the ADH A_2 enzyme protein was next investigated by starch gel electrophoresis to support PCR findings. All preferrers and avoiders yielded an ADH A_2 enzyme band and the band was absent in the *P. maniculatus* ADH^o liver extracts. Results from PCR and the starch gel experiments are consistent with earlier studies which found that both the ADH A_2 gene (Zheng *et al.*, 1993) and protein product (Burnett and Felder, 1978) are absent in ADH^o *P. maniculatus*. Because both the gene and protein product are present in the twelve *P. leucopus* tested, we have concluded that the

Peromyscus ADH^{o} phenotype is not the underlying cause of avoidance or preference in *P. leucopus*.

In *Peromyscus*, the ADH A_2 enzyme has been reported to be encoded by one locus with three identified alleles (Burnett and Felder, 1978). Two alleles encode electrophoretic fast and slow phenotype variants of the enzyme and the third is the ADH^{o} phenotype (Burnett and Felder, 1978). To investigate whether there may be a correlation between enzyme variant and preference phenotype in *P. leucopus*, liver extracts were run on starch gels to demonstrate banding patterns. It was found that four of the twelve animals studied possessed a single fast migrating band similar to the homozygote *P. maniculatus* Adh^{F}/Adh^{F} phenotype. The other eight possessed a double banding pattern similar to the heterozygote banding pattern Burnett and Felder (1978) described. Interestingly, the heterozygote *Peromyscus* banding pattern Burnett and Felder (1978) described resulted from a cross of *P. polionotus* which expresses only a slow migrating band and the *P. maniculatus* Adh^{F}/Adh^{F} phenotype.

When comparing all animals, however, this experiment shows no apparent correlation between ethanol preference and enzyme phenotype since 3 avoiders and 1 preferrer have the fast phenotype and the remaining 3 avoiders and 5 preferrers have the heterozygote banding pattern. Additionally, activity analysis of liver extracts presented in this study show no significant differences between the animals with the fast band and those with the heterozygote banding pattern. Not enough is known about the ADH enzyme system in *P. leucopus* to determine what variants may make up the observed banding patterns.

Since avoidance in *P. leucopus* does not seem to be caused by either the lack of the ADH A_2 enzyme (ADH^o phenotype) or a variant of the enzyme, the specific level of liver ADH activity was investigated. Initially, a semiquantitative method based upon intensity of band staining was used to determine if there were differing levels of ADH activity. A single gel was run with all extracts, photographed, and analyzed with NIH image analysis software. This experiment indicated that the mean density of the bands of the preferrers were significantly more dense ($P = 0.002$) than those of avoiders. Additionally, the darkest staining band belongs to animal 209 which is a preferrer and the lightest staining band belongs to animal 325 which is an avoider.

The same starch gel experiment also indicated that when the single fast migrating bands mentioned earlier were compared, the band from three animals (325, 224, and 227) were approximately half as dark as the band from animal 209. This difference in darkness is similar to that demonstrated by Burnett and Felder (1978) when comparing the heterozygote Adh^F/Adh^N phenotype to the much darker Adh^F/Adh^F phenotype. By investigating the specific ADH activity in the livers of these two phenotypes, Burnett and Felder (1978) found the activity of the heterozygote Adh^F/Adh^N phenotype to be approximately half that of the homozygote Adh^F/Adh^F phenotype. Since large activity differences may be present between these animals, it may hypothesized that there may be a difference in preference also. Indeed, animals 325, 224, and 227 are all avoiders and animal 209 is a preferrer.

To determine if differences in activity actually existed between these samples, specific liver ADH activity was measured in liver extracts from eight avoider *P. leucopus*, six

preferrer *P. leucopus* and two ADH° *P. maniculatus*. We found the average ADH activity in the livers of animals 325, 224, and 227 to be 14.5 (uM NADH/min/20 ul extract) compared to animal 209 who had an liver activity of 28.5 (uM NADH/min/20 ul extract). These activity differences are almost identical to comparisons between the Adh^F/Adh^N and Adh^F/Adh^F phenotype Burnett and Felder (1978) described. These findings demonstrate that the Adh^F/Adh^N phenotype cannot be dismissed as one possible underlying cause of ADH activity differences in *P. leucopus*.

Liver extracts from the two *P. maniculatus* had almost no ADH activity. Burnett and Felder (1978) found similar ADH activity when testing these animals. Since all ADH° *P. maniculatus* tested were determined previously to avoid an ethanol solution, these results support our hypothesis that avoidance is associated with compromised ethanol metabolism.

Importantly, activity tests also indicate *P. leucopus* avoiders have approximately 80% of the ADH liver activity that the preferrers have. These differences are significant and support our hypothesis that compromised ethanol metabolism is likely an underlying cause of avoidance in *Peromyscus*. Similar patterns of ADH activity have been found when comparing inbred strains of *Mus musculus* which prefer and avoid an ethanol solution (Rex *et al.*, 1984). The DBA inbred strain of *Mus musculus* avoid an ethanol solution and have approximately 50% of the liver ADH activity that the preferring C57BL inbred strain has (Balak *et al.*, 1982). We also tested these two mice strains to validate our tests and found the DBA mice had approximately 65% of the liver ADH activity that the male and female C57BL mice had. Direct comparisons with other studies can not be made because

of differing testing conditions. Ethanol avoiders with low liver ADH activity have also been identified in some rat strains (Koivisto and Eriksson, 1994). These results indicate that our activity analysis test is reliable.

When random selected *P. leucopus* were tested for ADH activity, the group mean was found to be intermediate between the mean values of preferrers and avoiders. These findings suggest that selection may be acting in part on ADH activity of these animals. There are, however, no significant differences when comparing the scores of the preferrers and avoiders to the random group. These observations are likely due to the large variation noted in the random tested animals from an outbred population. It is also interesting that the distribution of scores from the random group includes scores from both the preferrers and avoiders but there is little overlap between the two preference groups.

The underlying causes of these activity differences has not been clearly defined. Investigators have demonstrated that differences in inducibility of ADH is not likely the underlying cause of preference in *Mus musculus* by measuring mRNA levels after being fed ethanol for several days (Tagliabracci and Singh, 1996). Inducibility also does not correlate with liver ADH activity in *Mus musculus* (Wang and Singh, 1984). Specific enzyme catalytic activity differences are also unlikely since the purified enzymes from strains of *Mus musculus* which differ in liver ADH activity have the same enzyme characteristics such as Km and heat stability (Rex *et al.*, 1984); (Wang and Singh, 1984); (Zhang *et al.*, 1987). The final answer may lie in differing genetic-based levels of ADH. Indeed, (Balak *et al.*, 1982) has concluded that this is the underlying cause of ADH

activity differences between C57BL and BALB inbred mice but such findings have not been demonstrated in other animal models.

Initially, this study demonstrates that avoidance of 10% ethanol drinking solution seems to have a genetic basis in *P. leucopus*. With these findings in hand, we hypothesized that compromised ethanol metabolism may be an underlying cause of avoidance these mice. Since the ADH A_2 enzyme is the primary enzyme involved in ethanol metabolism (Vallee, 1985; Jornvall, 1994) and is missing in another species of *Peromyscus* (Burnett and Felder, 1978) we investigated its presence or absence in *P. leucopus*. After several experiments, we concluded that the ADH^o phenotype is not present in the avoiders and preferrers we tested and is therefore not the underlying cause of avoidance in *P. leucopus*.

These findings did not rule out the possibility that differing levels of ADH activity may be present and influence ethanol ingestion in these mice. Testing the ADH activities in liver extracts of 6 avoiders and 6 preferrers demonstrated that significant differences are present between these groups. Indeed, avoiders were shown to have less ADH activity thereby supporting our hypothesis that compromised ethanol metabolism may be an underlying cause of avoidance in *P. leucopus*. Additionally, we found that when 6 ADH^o *P. maniculatus* were subjected to the ethanol preference test, they all exhibited the avoider phenotype. These results also support our hypothesis that compromised ethanol metabolism is an underlying cause of avoidance in these mice. The reasons for these genetic differences in ADH activity were not clarified here and warrant further investigation.

In the future, several experiments should be undertaken to discover information concerning both the genetics of ethanol preference and ADH regulation in *Peromyscus*. More breeding experiments with *P. leucopus* avoiders and preferrers should be undertaken in hopes of identifying the heritability of the traits as well as the number of loci which may be involved. Once larger numbers of animals have been produced, attempts should be made to identify what types of ADH enzyme phenotypes may be present (such as the ADH^{o}) and true-breeding lines should be established and tested for ethanol preference. *P. maniculatus* avoiders randomly selected from the population also should be tested for the presence of the ADH^{o} phenotype.

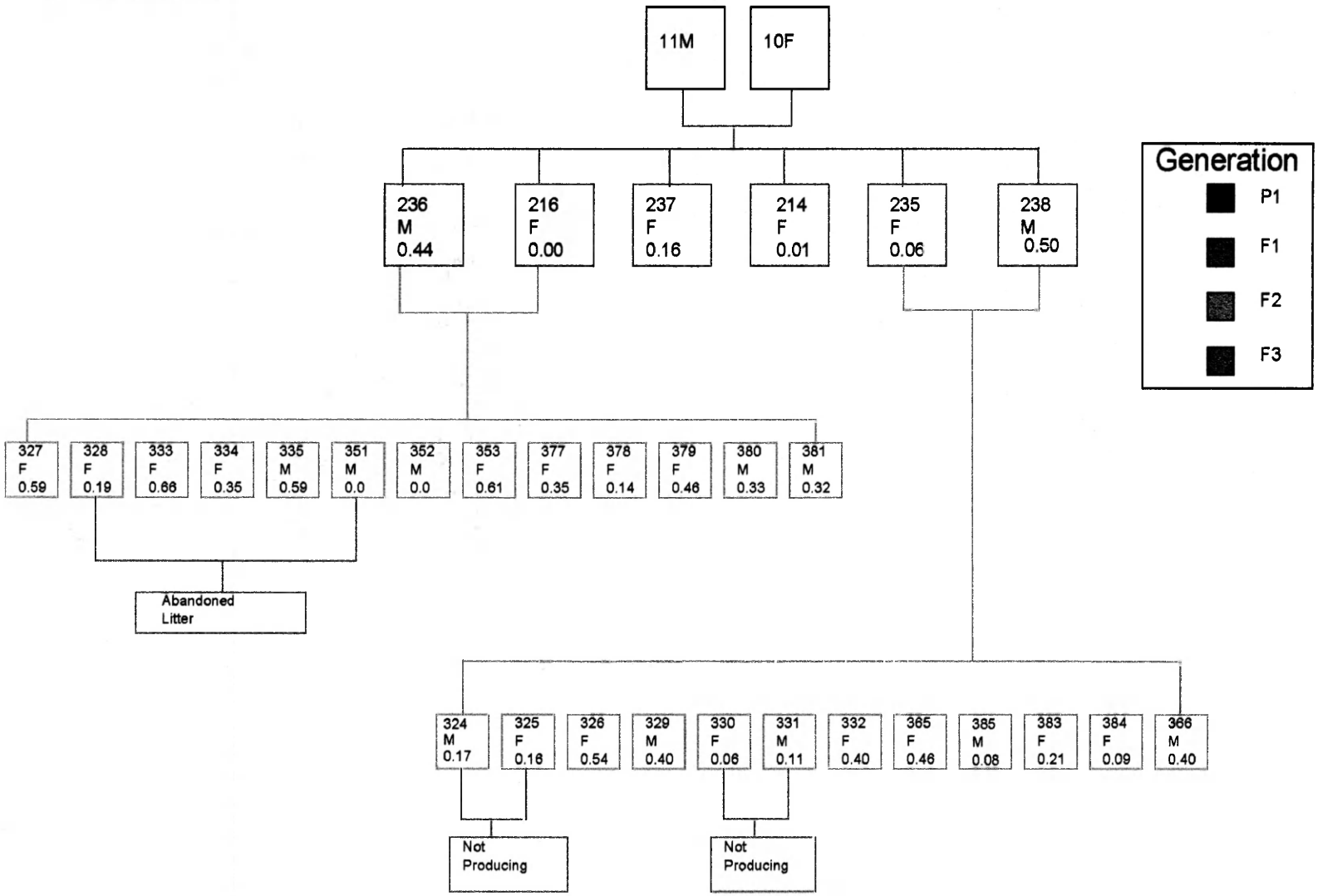
On the molecular level, sequencing of intron 1 in both preferrers and avoiders may yield interesting results since Zhang *et al.* (1987) found a 101 Bp deletion in strains of mice which avoid an ethanol solution and have low liver ADH activity. Investigating if differing levels of ADH protein synthesis may exist between avoiders and preferrers using *in vivo* radiolabeling and specific immunoprecipitation techniques may also yield some interesting results.

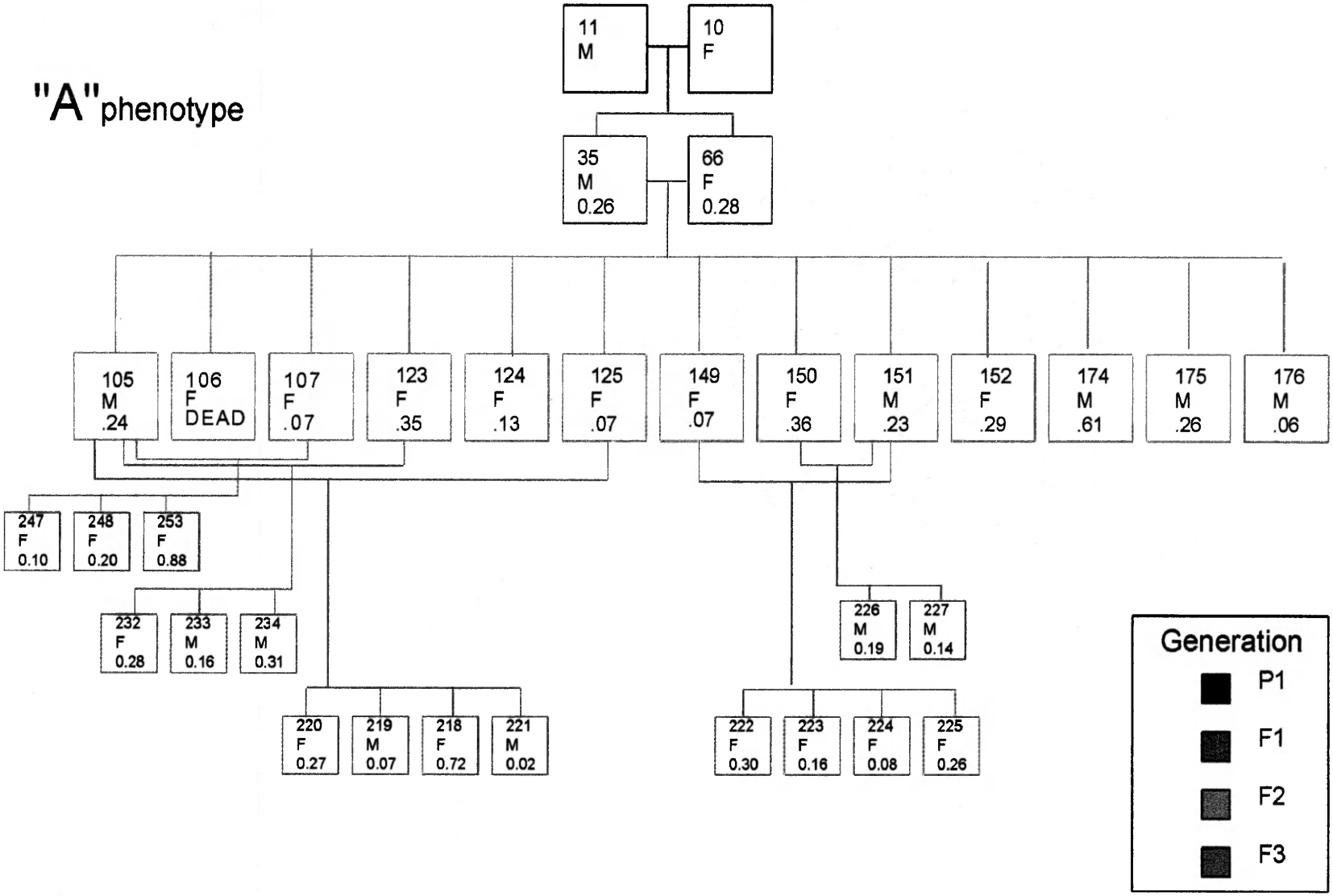
The findings of the present study indicate the possibility that differing genetic levels of ADH may exist and have an effect on ethanol preference in *Peromyscus*. In humans, where the complexity of class I ADH is extensive, higher catalytic properties of specific polymorphisms have been identified in people who also have a higher likelihood of avoiding ethanol (Shibuya *et al.*, 1989). On the other hand, attempts to investigate different genetic levels of the same enzyme have not been systematically explored and could yield interesting results. One major step in elucidating the underlying causes of

activity differences in mammals is identifying the mechanisms which control ADH expression. These transcriptional control mechanisms of ADH have not been clarified to date. Mammalian models, such as *P. leucopus*, which may have differing levels of ADH expression can provide valuable information concerning this enzymes regulation and perhaps its contribution to alcoholism. Furthermore, fully characterizing a mammalian model which seems to have a range of ethanol preference phenotypes may be beneficial to the broader investigation of underlying causes of alcoholism in humans.

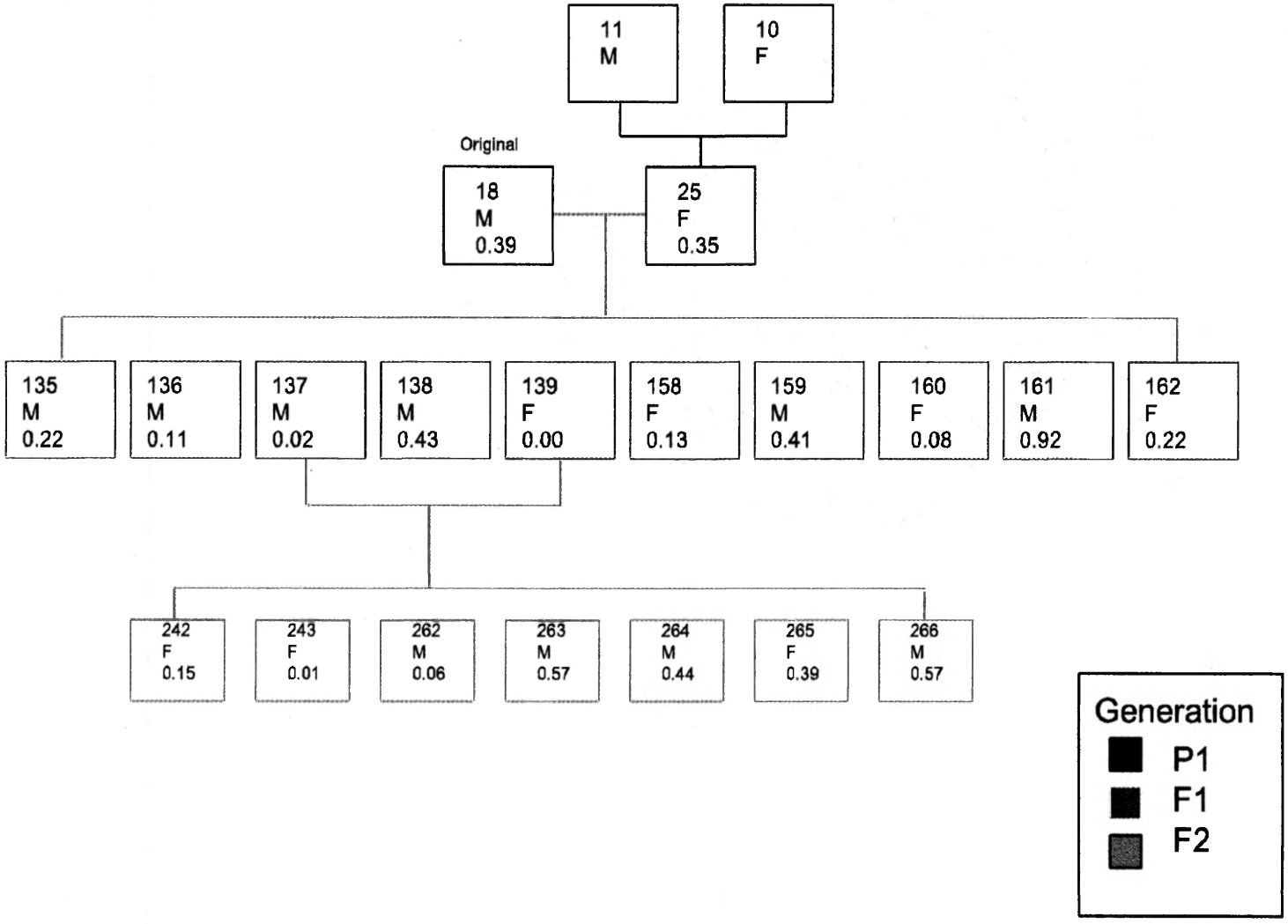
Appendix 1. Pedigrees for the F_1, F_2, and F_3 generations. The number identifies the specific animal, M or F identifies male or female, decimal number is EPR score.

"A" phenotype


11M
10F
236 M 0.44
216 F 0.00
237 F 0.16
214 F 0.01
235 F 0.06
238 M 0.50
Generation
P1
F1
F2
F3
327 F 0.59
328 F 0.19
333 F 0.66
334 F 0.35
335 M 0.59
351 M 0.0
352 M 0.0
353 F 0.61
377 F 0.35
378 F 0.14
379 F 0.46
380 M 0.33
381 M 0.32
Abandoned Litter
324 M 0.17
325 F 0.16
326 F 0.54
329 M 0.40
330 F 0.06
331 M 0.11
332 F 0.40
365 F 0.46
385 M 0.08
383 F 0.21
384 F 0.09
366 M 0.40
Not Producing
Not Producing


"A" phenotype
11
M
10
F
35
M
0.26
66
F
0.28
105
M
.24
106
F
DEAD
107
F
.07
123
F
.35
124
F
.13
125
F
.07
149
F
.07
150
F
.36
151
M
.23
152
F
.29
174
M
.61
175
M
.26
176
M
.06
247
F
0.10
248
F
0.20
253
F
0.88
232
F
0.28
233
M
0.16
234
M
0.31
220
F
0.27
219
M
0.07
218
F
0.72
221
M
0.02
226
M
0.19
227
M
0.14
222
F
0.30
223
F
0.16
224
F
0.08
225
F
0.26
Generation
P1
F1
F2
F3

"A"phenotype


11
M
10
F
Original
18
M
0.39
25
F
0.35
135
M
0.22
136
M
0.11
137
M
0.02
138
M
0.43
139
F
0.00
158
F
0.13
159
M
0.41
160
F
0.08
161
M
0.92
162
F
0.22
242
F
0.15
243
F
0.01
262
M
0.06
263
M
0.57
264
M
0.44
265
F
0.39
266
M
0.57
Generation
P1
F1
F2

Appendix 2. National Library of Medicine Blast search results from exon 5 and 6. Only the 15 best matches are presented. Query sequence is *P. leucopus* sequence determined in this study. Subject sequence refers to each of the 15 species compared.

15 best matches using Blast Search for exon 5

Sequences producing High-scoring Segment Pairs:	Smallest Sum Probability P(N)	N
Peromyscus maniculatus alcohol dehyd...	1.3e-56	2
Mouse adh-1 gene encoding alcohol de...	2.6e-43	2
Mouse alcohol dehydrogenase class I ...	2.8e-43	2
Mouse class I alcohol dehydrogenase ...	1.8e-42	2
Rat alcohol dehydrogenase (ADH) gene...	2.2e-41	2
Rat alcohol dehydrogenase (ADH) mRNA...	1.6e-39	2
O.cuniculus Adh1 mRNA for alcohol de...	4.8e-38	2
Horse alcohol dehydrogenase-E-isoenz...	2.0e-36	2
Human ADH2 (allele 2) gene for alcoh...	6.9e-36	2
Horse alcohol dehydrogenase-S-isoenz...	1.1e-35	2
Homo sapiens alcohol dehydrogenase b...	2.5e-35	2
Human alcohol dehydrogenase 3 gene, ...	3.6e-35	2
Human class I alcohol dehydrogenase ...	5.5e-35	2
Homo sapiens mRNA for alcohol dehydr...	8.6e-35	2
Human mRNA for alcohol dehydrogenase...	8.7e-35	2

gb|L15703|PERADH1B Peromyscus maniculatus alcohol dehydrogenase 1 (Adh-1) mRNA, complete cds.
Length = 1297

Plus Strand HSPs:

Score = 330 (91.2 bits), Expect = 1.3e-56, Sum P(2) = 1.3e-56
Identities = 66/66 (100%), Positives = 66/66 (100%), Strand = Plus / Plus

```
Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   430 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 489

Query:    61 CCCAGT 66
             ||||||
Sbjct:   490 CCCAGT 495
```

Score = 543 (150.0 bits), Expect = 1.3e-56, Sum P(2) = 1.3e-56
Identities = 111/114 (97%), Positives = 111/114 (97%), Strand = Plus / Plus

```
Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   497 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 556

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   557 CTGCCTCATAGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 610
```

gb|M18476|MUSADH1A5 Mouse adh-1 gene encoding alcohol dehydrogenase (ADH), exon 5.
Length = 250

Plus Strand HSPs:

Score = 240 (66.3 bits), Expect = 2.6e-43, Sum P(2) = 2.6e-43
Identities = 56/66 (84%), Positives = 56/66 (84%), Strand = Plus / Plus

```
Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||||||||||  ||||||  || |||   || ||||||||  ||||||||||||||  ||||
Sbjct:    51 CCAGCAGGTTCTCCTGCAAGGGAAAGCAGATCCACAACTTTATCAGCACCAGCACCTTCT 110

Query:    61 CCCAGT 66
             ||||||
Sbjct:   111 CCCAGT 116
```

Score = 462 (127.7 bits), Expect = 2.6e-43, Sum P(2) = 2.6e-43
Identities = 102/114 (89%), Positives = 102/114 (89%), Strand = Plus / Plus

```
Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||||||||| || |||||||| |||||||||| ||||||||||||| |||||
Sbjct:   118 CACCGTGGTAGATGATATAGCAGTGGCCAAAATCGATGGAGCTTCACCACTGGACAAAGT 177

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  |||||||||||  |||||||||||||  || ||||||||  ||||||||||
Sbjct:   178 CTGCCTCATCGGCTGTGGGTTCTCAACTGGCTATGGCTCTGCCGTCAAAGTCGC 231
```

gb|M22675|MUSADHA206 Mouse alcohol dehydrogenase class I (ADH-A-2) gene, exon 5.
Length = 261

Plus Strand HSPs:

Score = 240 (66.3 bits), Expect = 2.8e-43, Sum P(2) = 2.8e-43
Identities = 56/66 (84%), Positives = 56/66 (84%), Strand = Plus / Plus

```
Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||||||||||| ||||||  || |||   || ||||||||| ||||||||||||||| ||||
Sbjct:    56 CCAGCAGGTTCTCCTGCAAGGGAAAGCAGATCCACAACTTTATCAGCACCAGCACCTTCT 115

Query:    61 CCCAGT 66
             ||||||
Sbjct:   116 CCCAGT 121
```

Score = 462 (127.7 bits), Expect = 2.8e-43, Sum P(2) = 2.8e-43
Identities = 102/114 (89%), Positives = 102/114 (89%), Strand = Plus / Plus

```
Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| |||||||||||| || |||||||| |||||||||| |||||||||||||| |||||
Sbjct:   123 CACCGTGGTAGATGATATAGCAGTGGCCAAAATCGATGGAGCTTCACCACTGGACAAAGT 182

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  |||||||||||| |||||||||||||| || |||||||| ||||||||||
Sbjct:   183 CTGCCTCATCGGCTGTGGGTTCTCAACTGGCTATGGCTCTGCCGTCAAAGTCGC 236
```

gb|M11307|MUSADHIA Mouse class I alcohol dehydrogenase (ADH-AA) mRNA, complete cds.
Length = 1367

Plus Strand HSPs:

Score = 240 (66.3 bits), Expect = 1.8e-42, Sum P(2) = 1.8e-42
Identities = 56/66 (84%), Positives = 56/66 (84%), Strand = Plus / Plus

```
Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||||||||||| ||||||  || |||   || ||||||||| ||||||||||||||| ||||
Sbjct:   484 CCAGCAGGTTCTCCTGCAAGGGAAAGCAGATCCACAACTTTATCAGCACCAGCACCTTCT 543

Query:    61 CCCAGT 66
             ||||||
Sbjct:   544 CCCAGT 549
```

Score = 462 (127.7 bits), Expect = 1.8e-42, Sum P(2) = 1.8e-42
Identities = 102/114 (89%), Positives = 102/114 (89%), Strand = Plus / Plus

```
Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| |||||||||||| || |||||||| |||||||||| |||||||||||||| |||||
Sbjct:   551 CACCGTGGTAGATGATATAGCAGTGGCCAAAATCGATGGAGCTTCACCACTGGACAAAGT 610

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  |||||||||||| |||||||||||||| || |||||||| ||||||||||
Sbjct:   611 CTGCCTCATCGGCTGTGGGTTCTCAACTGGCTATGGCTCTGCCGTCAAAGTCGC 664
```

gb|M29519|RATADHX4 Rat alcohol dehydrogenase (ADH) gene, exons 4 and 5.
Length = 586

Plus Strand HSPs:

Score = 267 (73.8 bits), Expect = 2.2e-41, Sum P(2) = 2.2e-41
Identities = 59/66 (89%), Positives = 59/66 (89%), Strand = Plus / Plus

```
Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||||||||||| ||||||| || ||| | |||||| |||||||||||||||||| ||||
Sbjct:   370 CCAGCAGGTTCTCCTGCAGGGGAAAGCCCATTCACCACTTCATCAGCACCAGCACCTTCT 429

Query:    61 CCCAGT 66
             ||||||
Sbjct:   430 CCCAGT 435
```

Score = 417 (115.2 bits), Expect = 2.2e-41, Sum P(2) = 2.2e-41
Identities = 97/114 (85%), Positives = 97/114 (85%), Strand = Plus / Plus

```
Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             |||||||||||||||| || || ||||| |||||||||| |||| |||| ||||| |||||
Sbjct:   437 CACTGTGGTAGATGACATAGCGGTGGCCAAAATCGATGCGGCTGCACCGCTGGACAAAGT 496
```

```
Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  |||||||||||| || ||||| ||||| || |||||||| ||| |||||||
Sbjct:   497 CTGCCTCATCGGCTGTGGCTTCTCGACTGGCTATGGCTCTGCCGTCCAAGTCGC 550


gb|M15327|RATADH</b></a> Rat alcohol dehydrogenase (ADH) mRNA, complete cds.
           Length = 1292

  Plus Strand HSPs:

 Score = 240 (66.3 bits), Expect = 1.6e-39, Sum P(2) = 1.6e-39
 Identities = 56/66 (84%), Positives = 56/66 (84%), Strand = Plus / Plus

Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             ||||||| ||| ||||||| || ||| | |||||| |||| ||||||||||||| ||||
Sbjct:   420 CCAGCAGATTCTCCTGCAGGGGAAAGCCCATTCACCACTTTCTCAGCACCAGCACCTTCT 479

Query:    61 CCCAGT 66
             ||||||
Sbjct:   480 CCCAGT 485

 Score = 426 (117.7 bits), Expect = 1.6e-39, Sum P(2) = 1.6e-39
 Identities = 98/114 (85%), Positives = 98/114 (85%), Strand = Plus / Plus

Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             |||||||||||||||| || || ||||| |||||||||| |||| |||||||||| |||||
Sbjct:   487 CACTGTGGTAGATGACATAGCGGTGGCCAAAATCGATGCGGCTGCACCACTGGACAAAGT 546

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  |||||||||||| || ||||| ||||| || |||||||| ||| |||||||
Sbjct:   547 CTGCCTCATCGGCTGTGGCTTCTCGACTGGCTATGGCTCTGCCGTCCAAGTCGC 600


emb|X69799|OCADH1AA</b></a> O.cuniculus Adh1 mRNA for alcohol dehydrogenase, class I
           Length = 1296

  Plus Strand HSPs:

 Score = 249 (68.8 bits), Expect = 4.8e-38, Sum P(2) = 4.8e-38
 Identities = 57/66 (86%), Positives = 57/66 (86%), Strand = Plus / Plus

Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             ||||||||||||||||||  |||||| | || ||| |||||||  |||||||||| ||||
Sbjct:   418 CCAGCAGGTTCACCTGCAAGGGGAAGCCCATCCACCACTTCATTGGCACCAGCACCTTCT 477

Query:    61 CCCAGT 66
             ||||||
Sbjct:   478 CCCAGT 483

 Score = 399 (110.3 bits), Expect = 4.8e-38, Sum P(2) = 4.8e-38
 Identities = 95/114 (83%), Positives = 95/114 (83%), Strand = Plus / Plus

Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||| |||||||| |||||||| ||||| |||| ||| ||||||||||||||||
Sbjct:   485 CACAGTGGTGGATGAGATTGCAGTGGCCAAAATTGATGCAGCTGCACCACTGGAGAAAGT 544

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||||| ||||| || || |||||||||| || || || |||||||| ||
Sbjct:   545 CTGCCTCATTGGCTGTGGATTTTCAACTGGTTATGGGTCGGCAGTCAAAGTTGC 598


gb|M64864|HRSADHE</b></a> Horse alcohol dehydrogenase-E-isoenzyme mRNA, complete cds.
           Length = 1761

  Plus Strand HSPs:

 Score = 258 (71.3 bits), Expect = 2.0e-36, Sum P(2) = 2.0e-36
 Identities = 58/66 (87%), Positives = 58/66 (87%), Strand = Plus / Plus

Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             ||||||||||||||||||||||||| | || ||| ||||| |  |||||||||| ||||
Sbjct:   395 CCAGCAGGTTCACCTGCAGAGGGAAGCCCATCCACCACTTCCTTGGCACCAGCACCTTCT 454

Query:    61 CCCAGT 66
             ||||||
Sbjct:   455 CCCAGT 460

 Score = 372 (102.8 bits), Expect = 2.0e-36, Sum P(2) = 2.0e-36
 Identities = 92/114 (80%), Positives = 92/114 (80%), Strand = Plus / Plus
```

```
Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||| || |||||  ||||||| || |||||||| ||| ||||| ||||||||||
Sbjct:   462 CACCGTGGTGGACGAGATCTCAGTGGCCAAGATCGATGCGGCCTCACCGCTGGAGAAAGT 521

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||||| ||||| || || || |||||||| || ||||| ||||| || ||
Sbjct:   522 CTGTCTCATTGGCTGTGGATTTTCTACTGGTTATGGGTCTGCAGTCAAGGTTGC 575


emb|X15451|HSADH225</b></a> Human ADH2 (allele 2) gene for alcohol
           dehydrogenase beta-2 exon 5 (EC 1.1.1.1) gb|M24312|HUMADH2E5 Human
           class I alcohol dehydrogenase (ADH2) beta-1 subunit, allele 1 gene,
           exon 5.
           Length = 250

  Plus Strand HSPs:

 Score = 249 (68.8 bits), Expect = 6.9e-36, Sum P(2) = 6.9e-36
 Identities = 57/66 (86%), Positives = 57/66 (86%), Strand = Plus / Plus

Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||| |||||||||||||| |||||| | |||||| ||||| |  |||||||||| ||||
Sbjct:    51 CCAGGAGGTTCACCTGCAGGGGGAAGCCCATTCACCACTTCCTTGGCACCAGCACCTTCT 110

Query:    61 CCCAGT 66
             ||||||
Sbjct:   111 CCCAGT 116

 Score = 363 (100.3 bits), Expect = 6.9e-36, Sum P(2) = 6.9e-36
 Identities = 91/114 (79%), Positives = 91/114 (79%), Strand = Plus / Plus

Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||| ||||||| ||||||| ||||| |||| || || || ||||||||||
Sbjct:   118 CACGGTGGTGGATGAGAATGCAGTGGCCAAAATTGATGCAGCCTCGCCCCTGGAGAAAGT 177

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||||| ||||| || ||||| |||||||| || ||||| || || || ||
Sbjct:   178 CTGCCTCATTGGCTGTGGATTCTCGACTGGTTATGGGTCTGCAGTTAACGTTGC 231


gb|M64865|HRSADHS</b></a> Horse alcohol dehydrogenase-S-isoenzyme mRNA, complete cds.
           Length = 1832

  Plus Strand HSPs:

 Score = 258 (71.3 bits), Expect = 1.1e-35, Sum P(2) = 1.1e-35
 Identities = 58/66 (87%), Positives = 58/66 (87%), Strand = Plus / Plus

Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||||||||||||||||||||||||| | || ||| ||||| |  |||||||||| ||||
Sbjct:   412 CCAGCAGGTTCACCTGCAGAGGGAAGCCCATCCACCACTTCCTTGGCACCAGCACCTTCT 471

Query:    61 CCCAGT 66
             ||||||
Sbjct:   472 CCCAGT 477

 Score = 363 (100.3 bits), Expect = 1.1e-35, Sum P(2) = 1.1e-35
 Identities = 91/114 (79%), Positives = 91/114 (79%), Strand = Plus / Plus

Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||| || |||||  ||||||| || |||||||| ||| ||||| ||||||||||
Sbjct:   479 CACCGTGGTGGACGAGATCTCAGTGGCCAAGATCGATGCGGCCTCACCGCTGGAGAAAGT 538

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||| | ||||| || || || |||||||| || ||||| ||||| || ||
Sbjct:   539 CTGTCTCGTTGGCTGTGGATTTTCTACTGGTTATGGGTCTGCAGTCAAGGTTGC 592


gb|L38286|HUMADH2S04</b></a> Homo sapiens alcohol dehydrogenase beta-3 subunit
           (ADH2-3) gene, exons 4-5
           Length = 747

  Plus Strand HSPs:

 Score = 249 (68.8 bits), Expect = 2.5e-35, Sum P(2) = 2.5e-35
 Identities = 57/66 (86%), Positives = 57/66 (86%), Strand = Plus / Plus

Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||| |||||||||||||| |||||| | |||||| ||||| |  |||||||||| ||||
Sbjct:   319 CCAGGAGGTTCACCTGCAGGGGGAAGCCCATTCACCACTTCCTTGGCACCAGCACCTTCT 378
```

```
Query:    61 CCCAGT 66
             ||||||
Sbjct:   379 CCCAGT 384
```

Score = 363 (100.3 bits), Expect = 2.5e-35, Sum P(2) = 2.5e-35
Identities = 91/114 (79%), Positives = 91/114 (79%), Strand = Plus / Plus

```
Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||| |||||||  |||||||| ||||| ||||  || || || ||||||||||
Sbjct:   386 CACGGTGGTGGATGAGAATGCAGTGGCCAAAATTGATGCAGCCTCGCCCCTGGAGAAAGT 445

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||||| ||||| || ||||| |||||||| || ||||| || || || ||
Sbjct:   446 CTGCCTCATTGGCTGTGGATTCTCGACTGGTTATGGGTCTGCAGTTAACGTTGC 499
```

dbj|D11064|HUMALDEX5 Human alcohol dehydrogenase 3 gene, exon 5
Length = 240

Plus Strand HSPs:

Score = 231 (63.8 bits), Expect = 3.6e-35, Sum P(2) = 3.6e-35
Identities = 55/66 (83%), Positives = 55/66 (83%), Strand = Plus / Plus

```
Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||| |||||||||||||| |||||| | || ||| ||||| || ||  |||||| ||||
Sbjct:    46 CCAGGAGGTTCACCTGCAGCGGGAAGCCCATCCACCACTTCGTCGGCGTCAGCACCTTCT 105

Query:    61 CCCAGT 66
             ||||||
Sbjct:   106 CCCAGT 111
```

Score = 372 (102.8 bits), Expect = 3.6e-35, Sum P(2) = 3.6e-35
Identities = 92/114 (80%), Positives = 92/114 (80%), Strand = Plus / Plus

```
Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||| |||||||  |||||||| ||||| ||||  || || || ||||||||||
Sbjct:   113 CACGGTGGTGGATGAGAATGCAGTGGCCAAAATTGATGCAGCCTCGCCCCTGGAGAAAGT 172

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||||| ||||| || || || |||||||| || ||||| |||||||| ||
Sbjct:   173 CTGCCTCATTGGCTGTGGATTTTCGACTGGTTATGGGTCTGCAGTCAAAGTTGC 226
```

gb|M24317|HUMADH2BA Human class I alcohol dehydrogenase (ADH2) beta-1
subunit mRNA, complete cds.
Length = 1618

Plus Strand HSPs:

Score = 249 (68.8 bits), Expect = 5.5e-35, Sum P(2) = 5.5e-35
Identities = 57/66 (86%), Positives = 57/66 (86%), Strand = Plus / Plus

```
Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||| |||||||||||||| |||||| | |||||| ||||| |  |||||||||| ||||
Sbjct:   413 CCAGGAGGTTCACCTGCAGGGGGAAGCCCATTCACCACTTCCTTGGCACCAGCACCTTCT 472

Query:    61 CCCAGT 66
             ||||||
Sbjct:   473 CCCAGT 478
```

Score = 363 (100.3 bits), Expect = 5.5e-35, Sum P(2) = 5.5e-35
Identities = 91/114 (79%), Positives = 91/114 (79%), Strand = Plus / Plus

```
Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||| |||||||  |||||||| ||||| ||||  || || || ||||||||||
Sbjct:   480 CACGGTGGTGGATGAGAATGCAGTGGCCAAAATTGATGCAGCCTCGCCCCTGGAGAAAGT 539

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||||| ||||| || ||||| |||||||| || ||||| || || || ||
Sbjct:   540 CTGCCTCATTGGCTGTGGATTCTCGACTGGTTATGGGTCTGCAGTTAACGTTGC 593
```

dbj|D00137|HUMADH21C Homo sapiens mRNA for alcohol dehydrogenase beta
1, complete cds
Length = 2485

Plus Strand HSPs:

Score = 249 (68.8 bits), Expect = 8.6e-35, Sum P(2) = 8.6e-35
Identities = 57/66 (86%), Positives = 57/66 (86%), Strand = Plus / Plus

```
Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||| |||||||||||||||| |||||| | |||||| ||||| |  ||||||||||| ||||
Sbjct:   403 CCAGGAGGTTCACCTGCAGGGGGAAGCCCATTCACCACTTCCTTGGCACCAGCACCTTCT 462

Query:    61 CCCAGT 66
             ||||||
Sbjct:   463 CCCAGT 468

 Score = 363 (100.3 bits), Expect = 8.6e-35, Sum P(2) = 8.6e-35
 Identities = 91/114 (79%), Positives = 91/114 (79%), Strand = Plus / Plus

Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||| |||||||  |||||||| ||||| ||||  || || || ||||||||||
Sbjct:   470 CACGGTGGTGGATGAGAATGCAGTGGCCAAAATTGATGCAGCCTCGCCCCTGGAGAAAGT 529

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||||| ||||| || ||||| |||||||| || ||||| || || || ||
Sbjct:   530 CTGCCTCATTGGCTGTGGATTCTCGACTGGTTATGGGTCTGCAGTTAACGTTGC 583


emb|X03350|HSADHIBR</b></a> Human mRNA for alcohol dehydrogenase beta-1-subunit
            (ADH1-2 allele)
            Length = 2532

  Plus Strand HSPs:

 Score = 249 (68.8 bits), Expect = 8.7e-35, Sum P(2) = 8.7e-35
 Identities = 57/66 (86%), Positives = 57/66 (86%), Strand = Plus / Plus

Query:     1 CCAGCAGGTTCACCTGCAGAGGGAAGGCAATTCACAACTTCATCAGCACCAGCACTTTCT 60
             |||| |||||||||||||| |||||| | |||||| ||||| |  |||||||||| ||||
Sbjct:   455 CCAGGAGGTTCACCTGCAGGGGGAAGCCCATTCACCACTTCCTTGGCACCAGCACCTTCT 514

Query:    61 CCCAGT 66
             ||||||
Sbjct:   515 CCCAGT 520

 Score = 363 (100.3 bits), Expect = 8.7e-35, Sum P(2) = 8.7e-35
 Identities = 91/114 (79%), Positives = 91/114 (79%), Strand = Plus / Plus

Query:    67 CACTGTGGTAGATGAGATGGCAGTGGCTAAAATCGATGGGGCTTCACCACTGGAGAAAGT 126
             ||| ||||| |||||||  |||||||| ||||| ||||  || || || ||||||||||
Sbjct:   522 CACGGTGGTGGATGAGAATGCAGTGGCCAAAATTGATGCAGCCTCGCCCCTGGAGAAAGT 581

Query:   127 CTCGCTCATCGGCTGCGGGTTCTCAACTGGTTACGGCTCTGCTGTCAAAGTCGC 180
             ||  ||||| ||||| || ||||| |||||||| || ||||| || || || ||
Sbjct:   582 CTGCCTCATTGGCTGTGGATTCTCGACTGGTTATGGGTCTGCAGTTAACGTTGC 635
```

15 best matches using Blast Search for ADH exon 6

Sequences producing High-scoring Segment Pairs:	Smallest Sum Probability P(N)	N
Peromyscus maniculatus alcohol deh...	5.0e-72	1
Mouse class I alcohol dehydrogenas...	4.0e-64	1
Mouse alcohol dehydrogenase class ...	4.0e-64	1
Mouse adh-1 gene encoding alcohol ...	4.0e-64	1
Rat alcohol dehydrogenase (ADH) ge...	2.2e-60	1
Rat alcohol dehydrogenase (ADH) mR...	2.2e-60	1
O.cuniculus Adh1 mRNA for alcohol ...	2.2e-54	1
Peromyscus maniculatus alcohol deh...	3.8e-52	1
Horse alcohol dehydrogenase-E-isoe...	6.8e-52	1
Horse alcohol dehydrogenase-S-isoe...	6.8e-52	1
Homo sapiens alcohol dehydrogenase...	8.2e-52	1
Human mRNA for alcohol dehydrogena...	8.2e-52	1
Homo sapiens mRNA for alcohol dehy...	8.2e-52	1
Human class I alcohol dehydrogenas...	8.2e-52	1
Human ADH2 gene (allele 2) for alc...	8.2e-52	1

gb|L15703|PERADH1B Peromyscus maniculatus alcohol dehydrogenase 1 (Adh-1) mRNA, complete cds.
Length = 1297

Plus Strand HSPs:

Score = 984 (271.9 bits), Expect = 5.0e-72, P = 5.0e-72
Identities = 200/204 (98%), Positives = 200/204 (98%), Strand = Plus / Plus

```
Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             |||||||||||||||||||||||||||||| |||||||||||||| ||||||||||||||
Sbjct:   615 GTGACCCCAGGCTCCACCTGTGCTGTGTTCGGCCTCGGAGGTGTCGGTCTGTCTGTGATC 674

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             |||||||||||| | ||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   675 ATTGGCTGTAAAGCGGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 734

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:   735 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 794

Query:   181 CCCATCCAGGAAGTACTCCAGGAA 204
             ||||||||||||||||||||||||
Sbjct:   795 CCCATCCAGGAAGTACTCCAGGAA 818
```

gb|M11307|MUSADHIA Mouse class I alcohol dehydrogenase (ADH-AA) mRNA, complete cds.
Length = 1367

Plus Strand HSPs:

Score = 889 (245.6 bits), Expect = 4.0e-64, P = 4.0e-64
Identities = 189/203 (93%), Positives = 189/203 (93%), Strand = Plus / Plus

```
Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             |||||||||||||||| ||||| |||||||||||||||||||| |||||||||| |||
Sbjct:   669 GTGACCCCAGGCTCCACATGTGCCGTGTTTGGCCTCGGAGGTGTCGGTCTGTCTGTCATC 728

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             |||||||||||| |||||||||| |||||||||| |||||||||||||||| ||||||
Sbjct:   729 ATTGGCTGTAAAGCAGCAGGAGCAGCCAGGATCATTGCTGTGGACATCAACAAGGACAAG 788

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||||| |||||||||||| |||||||||||||||||||||||||| |||||| |||||
Sbjct:   789 TTTGCCAAGGCCAAAGAGTTGGGTGCAACTGAGTGCATCAACCCTCAAGACTACAGCAAA 848

Query:   181 CCCATCCAGGAAGTACTCCAGGA 203
             ||||||||||||| ||||||||
Sbjct:   849 CCCATCCAGGAAGTTCTCCAGGA 871
```

gb|M22676|MUSADHA207 Mouse alcohol dehydrogenase class I (ADH-A-2) gene, exon 6.
Length = 301

Plus Strand HSPs:

```
 Score = 889 (245.6 bits), Expect = 4.0e-64, P = 4.0e-64
 Identities = 189/203 (93%), Positives = 189/203 (93%), Strand = Plus / Plus

Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             ||||||||||||||||| ||||| ||||||||||||||||||| |||||||||| |||
Sbjct:    21 GTGACCCCAGGCTCCACATGTGCCGTGTTTGGCCTCGGAGGTGTCGGTCTGTCTGTCATC 80

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             |||||||||||| |||||||||| |||||||||| |||||||||||||||| ||||||
Sbjct:    81 ATTGGCTGTAAAGCAGCAGGAGCAGCCAGGATCATTGCTGTGGACATCAACAAGGACAAG 140

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||||| |||||||||| |||||||||||||||||||||||||| |||||| |||||
Sbjct:   141 TTTGCCAAGGCCAAAGAGTTGGGTGCAACTGAGTGCATCAACCCTCAAGACTACAGCAAA 200

Query:   181 CCCATCCAGGAAGTACTCCAGGA 203
             ||||||||||||||| ||||||||
Sbjct:   201 CCCATCCAGGAAGTTCTCCAGGA 223


gb|M18477|MUSADH1A6</b></a> Mouse adh-1 gene encoding alcohol dehydrogenase
           (ADH), exon 6.
           Length = 282

  Plus Strand HSPs:

 Score = 889 (245.6 bits), Expect = 4.0e-64, P = 4.0e-64
 Identities = 189/203 (93%), Positives = 189/203 (93%), Strand = Plus / Plus

Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             ||||||||||||||||| ||||| |||||||||||||||||||| |||||||||| |||
Sbjct:    16 GTGACCCCAGGCTCCACATGTGCCGTGTTTGGCCTCGGAGGTGTCGGTCTGTCTGTCATC 75

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             |||||||||||| |||||||||| |||||||||| |||||||||||||||| ||||||
Sbjct:    76 ATTGGCTGTAAAGCAGCAGGAGCAGCCAGGATCATTGCTGTGGACATCAACAAGGACAAG 135

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||||| |||||||||| |||||||||||||||||||||||||| |||||| |||||
Sbjct:   136 TTTGCCAAGGCCAAAGAGTTGGGTGCAACTGAGTGCATCAACCCTCAAGACTACAGCAAA 195

Query:   181 CCCATCCAGGAAGTACTCCAGGA 203
             ||||||||||||||| ||||||||
Sbjct:   196 CCCATCCAGGAAGTTCTCCAGGA 218


gb|M29520|RATADHX5</b></a> Rat alcohol dehydrogenase (ADH) gene, exon 6.
           Length = 409

  Plus Strand HSPs:

 Score = 844 (233.2 bits), Expect = 2.2e-60, P = 2.2e-60
 Identities = 184/203 (90%), Positives = 184/203 (90%), Strand = Plus / Plus

Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             |||||||||||||||||||||||| |||||||||| ||||||||||||||||||||  ||
Sbjct:   122 GTGACCCCAGGCTCCACCTGTGCCGTGTTTGGCCTGGGAGGTGTTGGTCTGTCTGTCGTC 181

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             |||||||||||||||||||||||| |||| |||||| || ||||||||||||||||||||
Sbjct:   182 ATTGGCTGTAAAACAGCAGGAGCAGCCAAGATCATTGCCGTGGACATCAACAAAGACAAG 241

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||||| |||||||||| || ||||| ||||| || |||||||||| |||||| | |||
Sbjct:   242 TTTGCGAAGGCCAAAGAGTTAGGTGCCACTGACTGTATCAACCCTCAAGACTACACCAAA 301

Query:   181 CCCATCCAGGAAGTACTCCAGGA 203
             ||||||||||||||| ||||||||
Sbjct:   302 CCCATCCAGGAAGTTCTCCAGGA 324


gb|M15327|RATADH</b></a> Rat alcohol dehydrogenase (ADH) mRNA, complete cds.
           Length = 1292

  Plus Strand HSPs:

 Score = 844 (233.2 bits), Expect = 2.2e-60, P = 2.2e-60
 Identities = 184/203 (90%), Positives = 184/203 (90%), Strand = Plus / Plus
```

```
Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             ||||||||||||||||||||||||| |||||||||| |||||||||||||||||||  ||
Sbjct:   605 GTGACCCCAGGCTCCACCTGTGCCGTGTTTGGCCTGGGAGGTGTTGGTCTGTCTGTCGTC 664

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             |||||||||||||||||||||| |||| |||||| || ||||||||||||||||||||||
Sbjct:   665 ATTGGCTGTAAAACAGCAGGAGCAGCCAAGATCATTGCCGTGGACATCAACAAAGACAAG 724

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||||| |||||||||| || ||||| ||||| || |||||||||| |||||| | |||
Sbjct:   725 TTTGCGAAGGCCAAAGAGTTAGGTGCCACTGACTGTATCAACCCTCAAGACTACACCAAA 784

Query:   181 CCCATCCAGGAAGTACTCCAGGA 203
             ||||||||||||| ||||||||
Sbjct:   785 CCCATCCAGGAAGTTCTCCAGGA 807


emb|X69799|OCADH1AA</b></a> O.cuniculus Adh1 mRNA for alcohol dehydrogenase, class I
           Length = 1296

  Plus Strand HSPs:

 Score = 772 (213.3 bits), Expect = 2.2e-54, P = 2.2e-54
 Identities = 176/203 (86%), Positives = 176/203 (86%), Strand = Plus / Plus

Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             || ||||||||||||||||||||||||||||||||| ||||| ||||| || ||||| |||
Sbjct:   603 GTCACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTGGGAGGAGTTGGCCTCTCTGTTATC 662

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             || ||||||||| |||| || ||  |||||||||| || |||||||| ||||||||||||
Sbjct:   663 ATGGGCTGTAAAGCAGCTGGGGCATCCAGGATCATTGCAGTGGACATTAACAAAGACAAG 722

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||| ||||||||||||||  ||||||| ||||||||||||||||||| ||||||||  ||
Sbjct:   723 TTTCCAAAGGCCAAAGAGGTGGGTGCCACTGAGTGCATCAACCCTCAAGACTATAAGAAA 782

Query:   181 CCCATCCAGGAAGTACTCCAGGA 203
             |||||||||||  ||  | |||||
Sbjct:   783 CCCATCCAGGAGGTGATACAGGA 805

gb|L15704|PERADH2A</b></a> Peromyscus maniculatus alcohol dehydrogenase 2
           (Adh-2) mRNA, complete cds.
           Length = 1869

  Plus Strand HSPs:

 Score = 745 (205.9 bits), Expect = 3.8e-52, P = 3.8e-52
 Identities = 169/194 (87%), Positives = 169/194 (87%), Strand = Plus / Plus

Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             ||| | ||||||||||||||||||||||||||||| |||||||||||||||||||| |||
Sbjct:   703 GTGTCTCCAGGCTCCACCTGTGCTGTGTTTGGCCTTGGAGGTGTTGGTCTGTCTGTCATC 762

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             || ||||||||| |||||||||| |||||||||||||||||| ||||||||||||
Sbjct:   763 ATGGGCTGTAAAGCAGCAGGAGCAGCCAGGATCATCGCTGTGGACACCAACAAAGACAAG 822

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             |||||||||||||    | ||||||| |||||||||||  |||| | ||||| |   ||
Sbjct:   823 TTTGCAAAGGCCAAGACAGTGGGTGCCACTGAGTGCATAGACCCACAAGACTTTGAGAAA 882

Query:   181 CCCATCCAGGAAGT 194
             ||||| ||  ||||
Sbjct:   883 CCCATTCAACAAGT 896


gb|M64864|HRSADHE</b></a> Horse alcohol dehydrogenase-E-isoenzyme mRNA, complete cds.
           Length = 1761

  Plus Strand HSPs:

 Score = 742 (205.0 bits), Expect = 6.8e-52, P = 6.8e-52
 Identities = 170/197 (86%), Positives = 170/197 (86%), Strand = Plus / Plus

Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             || ||||  |||||||||||||| |||||||||| ||||| || || |||||||| |||
Sbjct:   580 GTCACCCAGGGCTCCACCTGTGCCGTGTTTGGCCTTGGAGGAGTGGGCCTGTCTGTTATC 639

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             || ||||||||| |||| |||||||||||||||| |  ||||||||||||||||||||
Sbjct:   640 ATGGGCTGTAAAGCAGCCGGAGCGGCCAGGATCATTGGGGTGGACATCAACAAAGACAAG 699
```

```
Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||||||||||||||||||| |||||||| ||||||||  |||||||||  ||||| |  ||
Sbjct:   700 TTTGCAAAGGCCAAAGAAGTGGGTGCCACTGAGTGTGTCAACCCTCAGGACTACAAGAAA 759

Query:   181 CCCATCCAGGAAGTACT 197
             ||||||||||| || ||
Sbjct:   760 CCCATCCAGGAGGTGCT 776
```

gb|M64865|HRSADHS Horse alcohol dehydrogenase-S-isoenzyme mRNA, complete cds.
Length = 1832

Plus Strand HSPs:

Score = 742 (205.0 bits), Expect = 6.8e-52, P = 6.8e-52
Identities = 170/197 (86%), Positives = 170/197 (86%), Strand = Plus / Plus

```
Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             || ||||  |||||||||||||| |||||||||| ||||| || || |||||||| |||
Sbjct:   597 GTCACCCAGGGCTCCACCTGTGCCGTGTTTGGCCTTGGAGGAGTGGGCCTGTCTGTTATC 656

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             || ||||||||| |||| |||||||||||||||| |  ||||||||||||||||||||
Sbjct:   657 ATGGGCTGTAAAGCAGCCGGAGCGGCCAGGATCATTGGGGTGGACATCAACAAAGACAAG 716

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             |||||||||||||||||| ||||||| ||||||||  |||||||||  ||||| |  ||
Sbjct:   717 TTTGCAAAGGCCAAAGAAGTGGGTGCCACTGAGTGTGTCAACCCTCAGGACTACAAGAAA 776

Query:   181 CCCATCCAGGAAGTACT 197
             ||||||||||| || ||
Sbjct:   777 CCCATCCAGGAGGTGCT 793
```

gb|L38287|HUMADH2S05 Homo sapiens alcohol dehydrogenase beta-3 subunit
(ADH2-3) gene, exon 6
Length = 315

Plus Strand HSPs:

Score = 741 (204.8 bits), Expect = 8.2e-52, P = 8.2e-52
Identities = 173/204 (84%), Positives = 173/204 (84%), Strand = Plus / Plus

```
Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             || |||||||||| ||||||||||||||||||| ||||| ||||| || ||||    |
Sbjct:    23 GTCACCCCAGGCTCTACCTGTGCTGTGTTTGGCCTGGGAGGGGTTGGCCTATCTGCTGTT 82

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             || ||||||||| |||| ||||| ||||| ||||| || ||||||||||||| |||||
Sbjct:    83 ATGGGCTGTAAAGCAGCTGGAGCAGCCAGAATCATTGCGGTGGACATCAACAAGGACAAA 142

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||||||||||||||||| |||||||| ||||| |||||||||||| |||||| |  ||
Sbjct:   143 TTTGCAAAGGCCAAAGAGTTGGGTGCCACTGAATGCATCAACCCTCAAGACTACAAGAAA 202

Query:   181 CCCATCCAGGAAGTACTCCAGGAA 204
             ||||| |||||||| ||  |||||
Sbjct:   203 CCCATTCAGGAAGTGCTAAAGGAA 226
```

emb|X03350|HSADHIBR Human mRNA for alcohol dehydrogenase beta-1-subunit
(ADH1-2 allele)
Length = 2532

Plus Strand HSPs:

Score = 741 (204.8 bits), Expect = 8.2e-52, P = 8.2e-52
Identities = 173/204 (84%), Positives = 173/204 (84%), Strand = Plus / Plus

```
Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             || |||||||||| |||||||||||||||||||| ||||| || || || ||||    |
Sbjct:   640 GTCACCCCAGGCTCTACCTGTGCTGTGTTTGGCCTGGGAGGGGTCGGCCTATCTGCTGTT 699

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             || ||||||||| |||| ||||| ||||| ||||| || ||||||||||||| |||||
Sbjct:   700 ATGGGCTGTAAAGCAGCTGGAGCAGCCAGAATCATTGCGGTGGACATCAACAAGGACAAA 759

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             |||||||||||||||| |||||||| ||||| |||||||||||| |||||| |  ||
Sbjct:   760 TTTGCAAAGGCCAAAGAGTTGGGTGCCACTGAATGCATCAACCCTCAAGACTACAAGAAA 819
```

```
Query:   181 CCCATCCAGGAAGTACTCCAGGAA 204
             ||||||||||||||| ||  |||||
Sbjct:   820 CCCATCCAGGAAGTGCTAAAGGAA 843
```

dbj|D00137|HUMADH21C Homo sapiens mRNA for alcohol dehydrogenase beta 1, complete cds
Length = 2485

Plus Strand HSPs:

Score = 741 (204.8 bits), Expect = 8.2e-52, P = 8.2e-52
Identities = 173/204 (84%), Positives = 173/204 (84%), Strand = Plus / Plus

```
Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             || |||||||||| |||||||||||||||||||| ||||| || || || ||||   |
Sbjct:   588 GTCACCCCAGGCTCTACCTGTGCTGTGTTTGGCCTGGGAGGGGTCGGCCTATCTGCTGTT 647

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             || ||||||||| |||| ||||| ||||| ||||| || ||||||||||||| |||||
Sbjct:   648 ATGGGCTGTAAAGCAGCTGGAGCAGCCAGAATCATTGCGGTGGACATCAACAAGGACAAA 707

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             ||||||||||||||||| |||||||| ||||| |||||||||||| |||||| |  ||
Sbjct:   708 TTTGCAAAGGCCAAAGAGTTGGGTGCCACTGAATGCATCAACCCTCAAGACTACAAGAAA 767

Query:   181 CCCATCCAGGAAGTACTCCAGGAA 204
             |||||||||||||| ||  |||||
Sbjct:   768 CCCATCCAGGAAGTGCTAAAGGAA 791
```

gb|M24313|HUMADH2E6 Human class I alcohol dehydrogenase (ADH2) beta-1 subunit, allele 1 gene, exon 6.
Length = 291

Plus Strand HSPs:

Score = 741 (204.8 bits), Expect = 8.2e-52, P = 8.2e-52
Identities = 173/204 (84%), Positives = 173/204 (84%), Strand = Plus / Plus

```
Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             || |||||||||| |||||||||||||||||||| ||||| || || || ||||   |
Sbjct:    16 GTCACCCCAGGCTCTACCTGTGCTGTGTTTGGCCTGGGAGGGGTCGGCCTATCTGCTGTT 75

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             || ||||||||| |||| ||||| ||||| ||||| || ||||||||||||| |||||
Sbjct:    76 ATGGGCTGTAAAGCAGCTGGAGCAGCCAGAATCATTGCGGTGGACATCAACAAGGACAAA 135

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             |||||||||||||||| |||||||| ||||| |||||||||||| |||||| |  ||
Sbjct:   136 TTTGCAAAGGCCAAAGAGTTGGGTGCCACTGAATGCATCAACCCTCAAGACTACAAGAAA 195

Query:   181 CCCATCCAGGAAGTACTCCAGGAA 204
             |||||||||||||| ||  |||||
Sbjct:   196 CCCATCCAGGAAGTGCTAAAGGAA 219
```

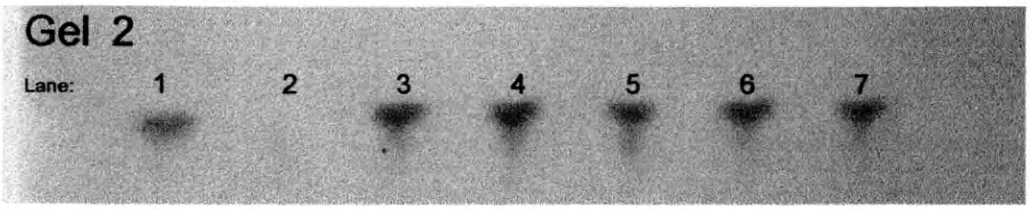
emb|X15452|HSADH226 Human ADH2 gene (allele 2) for alcohol dehydrogenase beta-2 exon 6 (EC 1.1.1.1)
Length = 291

Plus Strand HSPs:

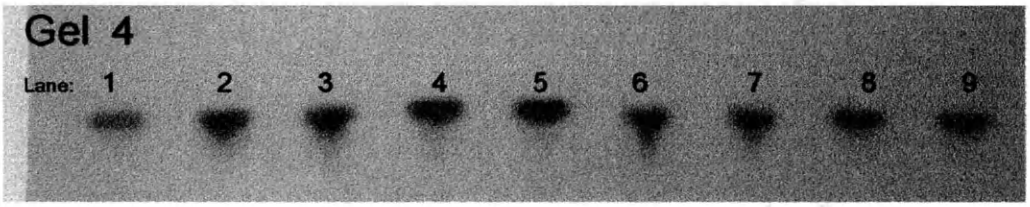
Score = 741 (204.8 bits), Expect = 8.2e-52, P = 8.2e-52
Identities = 173/204 (84%), Positives = 173/204 (84%), Strand = Plus / Plus

```
Query:     1 GTGACCCCAGGCTCCACCTGTGCTGTGTTTGGCCTCGGAGGTGTTGGTCTGTCTGTGATC 60
             || |||||||||| |||||||||||||||||||| ||||| || || || ||||   |
Sbjct:    16 GTCACCCCAGGCTCTACCTGTGCTGTGTTTGGCCTGGGAGGGGTCGGCCTATCTGCTGTT 75

Query:    61 ATTGGCTGTAAAACAGCAGGAGCGGCCAGGATCATCGCTGTGGACATCAACAAAGACAAG 120
             || ||||||||| |||| ||||| ||||| ||||| || ||||||||||||| |||||
Sbjct:    76 ATGGGCTGTAAAGCAGCTGGAGCAGCCAGAATCATTGCGGTGGACATCAACAAGGACAAA 135

Query:   121 TTTGCAAAGGCCAAAGAATTGGGTGCAACTGAGTGCATCAACCCTCTAGACTATAGCAAG 180
             |||||||||||||||| |||||||| ||||| |||||||||||| |||||| |  ||
Sbjct:   136 TTTGCAAAGGCCAAAGAGTTGGGTGCCACTGAATGCATCAACCCTCAAGACTACAAGAAA 195

Query:   181 CCCATCCAGGAAGTACTCCAGGAA 204
             |||||||||||||| ||  |||||
Sbjct:   196 CCCATCCAGGAAGTGCTAAAGGAA 219
```

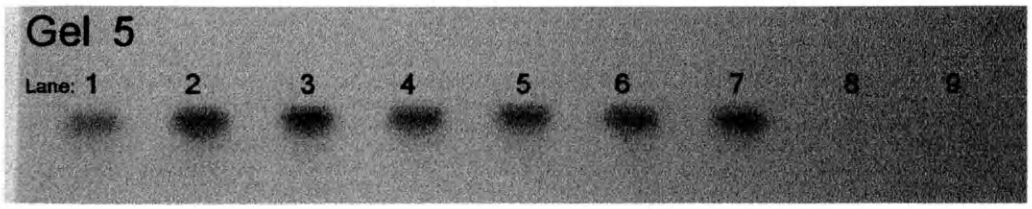
Appendix 3. Starch gel electrophoresis to determine the presence or absence of the ADH A_2 enzyme in preferrers and avoiders.



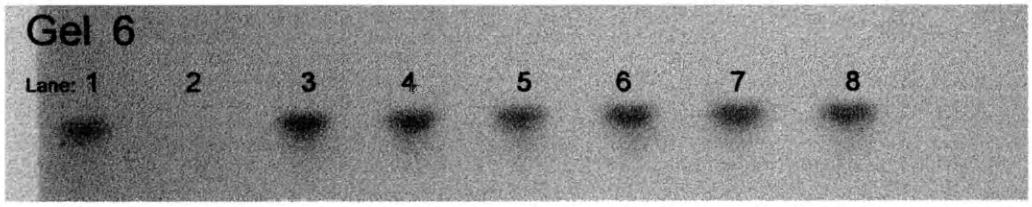
Lane	Animal
1	Control
2	ADH-
3	227
4	224
5	325
6	227
7	325



Lane	Animal
1	control
2	103
3	103
4	209
5	209
6	124
7	124
8	125
9	125



Lane	Animal
1	control
2	129
3	129
4	242
5	242
6	114
7	114
8	ADH-
9	ADH-



Lane	Animal
1	control
2	ADH-
3	111
4	111
5	111
6	119
7	119
8	119

Appendix 4. Results from enzyme activity analysis. Results are expressed as uM NADH /min/20 ul tissue extract.

Preferers	Preference	P	P	P	P	P	P	Mean	SD
(P. leucopus)	Animal identification	111	119	114	103	209	129	PREFER	Prefer
	uM NADH/min/20ul extract	16.1351	20.0751	18.0113	24.3902	28.5178	20.8255	21.3258	4.49131

Avoiders	Preference	A	A	A	A	A	A	A	A	Mean	SD
(P. leucopus)	Animal identification	124	325	233	226	224	227	125	242	AVOID	Avoid
	uM NADH/min/20ul extract	13.8837	10.8818	14.6341	19.8874	17.0732	15.5722	15.197	19.5122	15.8302	2.96977

Male and Female Avoiders	Preference	A	A	A	A	A	Mean	SD
(P. leucopus)	Animal identification	125	242	224	124	325	Female A	
	uM NADH/min/20ul extract	15.197	19.5122	17.0732	13.8837	10.8818	15.3096	3.25666

		A	A	A	Mean	SD
		227	233	226	Male A	
		15.5722	14.6342	19.8874	16.6979	2.80172

Control P.leucopus	Animal	C1	C2	C3	C4*	C5*	C6	C7	C8	Mean	SD
(P. leucopus)	uM NADH/min/20ul extract	29.268	17.073	26.079	24.953	8.63	12.383	22.514	11.257	19.76	7.37

Inbred Mice		C57BL-M	C57BL-F	Mean	DBA-M	DBA-F	Mean
(Mus musculus)	uM NADH/min/20ul extract	32.083	29.456	30.77	19.7	20.075	19.89

ADH negative mouse		Score
(P. maniculatus)	uM NADH/min/20ul extract	1.313

Isozyme types		fast			
(P. leucopus)	Animal identification	224	227	325	209
	uM NADH/min/20ul extract	17.0732	15.5722	10.88	28.5178

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